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The European Hematology Association (EHA) aims to promote excellence in clinical practice, research and education in European hematology. EHA was founded in June 1992 and today – with over 3500 members from 100 countries – is a consolidated representative of European hematologists.

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− To become the official European representative of hematology and hematologists – especially where research, education and regulatory issues are concerned – and to become a conduit for European harmonization;
− To promote the creation of a highly attractive market for practitioners and researchers in Europe thus fostering the mobility of hematologists in and to Europe;
− To reach out and offer a platform to countries that wish to further develop excellence in hematology;
− To promote education, training and scientific research in hematology in Europe;
− To exchange and disseminate knowledge and scientific information in the field of hematology.

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− Organizing an annual scientific and educational congress in a major European city;
− Dissemination of medical research, both basic and clinic, through the Haematologica/The Hematology Journal;
− Collaborating with other leading organizations in the field of hematology and oncology;
− Providing postgraduate education through the annual congress, tutorials and workshops;
− Supporting junior basic and clinical researchers in the development of their careers through the EHA Fellowship Program.
− Strengthening the quality and professional status of hematology throughout Europe by accrediting scientific meetings and providing CME accounts.

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On behalf of the EHA Board and the Scientific Program Committee we are pleased to present the Abstract Book of the 16th Congress of EHA.

The Scientific Program Committee has compiled an interesting program of Simultaneous Sessions and Poster Sessions from almost 2,000 submitted abstracts. Please join our expert moderators for a walk along the posters in your field of interest on Friday and Saturday. The six best abstracts have been selected for presentation during the Presidential Symposium on Saturday, June 11.

On behalf of the EHA Board, the committees and all the people involved in this year’s EHA congress, we thank you for coming to London and hope that this Abstract Book will provide you with an important reference source of recent advances in hematology research.

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EHA FUTURE CONGRESSES

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Amsterdam, The Netherlands
June 14-17 2012

18th CONGRESS
of the European Hematology Association
Stockholm, Sweden
June 13-16 2013

19th CONGRESS
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Milan, Italy
June 12-15 2014
Acute lymphoblastic leukemia - Biology

0001
RESEQUENCING OF 97 ONCogenES AND CANDidATE ONCOgenes IDENTIFIES TYK2 MUTATIONS IN T-ALL
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Background. T-cell acute lymphoblastic leukemia (T-ALL) is a malignancy of T-cell precursors that mainly occurs in children and adolescents. Optimization of chemotherapy regimens has resulted in improved responses with cure rates up to 75% in children. However, the current therapies are very toxic and cure rates rapidly decline in older patients, indicating the need for better and less toxic therapies. 

Methods. We sequenced the coding regions of 97 genes (known and candidate oncogenes/tumor suppressor genes) and subsequently sequenced these exons using 454 sequencing technology. In addition, all exons of the TYK2 gene were sequenced according to the Sanger method in diagnostic samples of 96 T-ALL patients; 54 acute lymphoblastic leukemia (ALL), we sequenced the coding regions of 97 genes in T-ALL cell lines and T-ALL samples. 

Results. Resequencing of these 97 genes in T-ALL cell lines and primary T-ALL cases identified mutations in known T-ALL associated oncogenes such as NOTCH1, FBXW7, PHF6, HRAS/NRAS/KRAS, PTEN, TP53, NF1, as well as novel mutations in genes encoding tyrosine kinases (JGFR, TYK2) and negative regulators of signaling such as tyrosine phosphatases (PTPDC) and sprouty related proteins (SPREDS). Remarkably, T-ALL cell lines were characterized by frequent mutation of TYK2 (6 of 18 T-ALL cell lines), a JAK kinase family member. While previous studies have described JAK1, JAK2, and JAK3 mutations in ALL patients, TYK2 mutations were previously not described in leukemia. Sequence analysis of TYK2 in primary patient samples identified TYK2 mutations in 2 of 97 T-ALL cases and 1 of 54 AML, but none of 53 B-ALL. Expression of TYK2 mutants in Ba/F3 and MOHITO cells conferred factor-independent growth, with some mutations being significantly more transforming than wild type TYK2, which by itself also transformed these lymphoid cell lines. Western blot analysis showed constitutive phosphorylation of TYK2, and also of JAK1 and STAT3, indicating that TYK2 mutants signal through JAK1 and STAT3. Overall, mutations in JAK1, JAK2, JAK3, and TYK2 were identified in approximately 15% of T-ALL cases. 

Conclusions. In addition to JAK1, JAK2 and JAK3 mutations, our data show that activating TYK2 mutations can also occur in acute leukemia. TYK2 mutations result in constitutive activation of the TYK2/JAK2/STAT3 pathway and represent an attractive new molecular target for JAK kinase inhibitors. These data show that the entire JAK kinase family can be mutated in acute leukemia, and warrant further clinical trials with JAK inhibitors for treatment of patients with JAK1, JAK2, JAK3 or TYK2 mutation.

0002
PTEN/AKT PATHWAY MUTATIONS ARE RECIPROCAL TO NOTCH1-ACTIVATING MUTATIONS IN PEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL)
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PTEN-inactivating mutations that affect the PI3K/akt pathway are often found in T-cell Acute Lymphoblastic Leukemia (T-ALL). To investigate the incidence of the PTEN/PI3K pathway aberrations in pediatric T-ALL, we retrospectively determined the incidence of PTEN, PI3K and AKT mutations in 142 pediatric T-ALL patients treated according to the Dutch DCOG or German COALL protocols. Using PCR-sequencing and array-CGH techniques, PTEN mutations/deletions were detected in 15% of the patients. In addition, we found three patients, of which one also carried a PTEN mutation, having aberrant PTEN splice products. Another 2% of the patients carried an AKT mutation, whereas no PI3K mutations were identified. Using reverse-phase protein microarray analysis (REMA), we demonstrated that PTEN protein expression was absent in PTEN-mutated or PTEN-deleted patients or patients with aberrant splicing, with the exception of one patient that carried a PTEN missense mutation. We also identified two T-ALL cases that lacked PTEN protein expression in the absence of PTEN mutations, defective splicing or for differential PTEN promoter hypermethylation in these patients. Therefore, 18% of the patients had PTEN or AKT aberrations in total. PTEN or AKT mutations (PTEN/akt) were especially observed in TAL or LMO-rearranged T-ALL patients (p=0.007), but not in HOX-rearranged cases (p=0.005). PTEN/akt mutations followed a pattern that seems reciprocal to the presence of NOTCH-activating mutations; they hardly co-express with NOTCH1-mutated mutations and PTEN/akt-mutated cells express low protein levels of activated NOTCH1, c-myc and MYC. This indicates that PTEN/akt mutations are not simply secondary events following NOTCH1/FBXW7 mutations such as initially reported. This also explains why secretase inhibitor insensitivity of PTEN-mutated cell lines, not observed in acquired resistance as consequence of oncogenic addiction switch1. Survival rates for PTEN/akt patients seemed comparable to wild-type patients (5-yrs DFS DCOG WT 71±6% vs PTEN/akt 70±16%, COALL WT 78±6% vs PTEN/akt 57±18%). However, when NOTCH1/FBXW7-mutated patients (associated with poor survival rates in DCOG and COALL cohorts) were separated from the wild-type group, the presence for NOTCH1/FBXW7 or PTEN/akt mutations was associated with poor outcome, whereas the disease-free survival rate for wild-type patients was nearly 100% (5-yrs DFS DCOG+COALL WT 92±6% vs PTEN/akt/NOTCH1 65±5%, p=0.005).

0003
FLOW CYTOMETRY AND IG/TCR QUANTITATIVE PCR FOR MINIMAL RESIDUAL DISEASE QUANTITATION IN ACUTE LYMPHOBLASTIC LEUKEMIA: A FRENCH MULTICENTER PROSPECTIVE STUDY
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Background. Being a strong prognostic factor in acute lymphoblastic leukemia (ALL), minimal residual disease (MRD) evaluation is now used for treatment stratification. Two methods are currently used: quantification of Ig/TCR clonal rearrangements using allele-specific quantitative PCR (Ig/TCR-QPCR) and multidimensional flow cytometry (FC). Ig/TCR-QPCR is now standardised and quality control rounds organised by the EuroMRD consortium ensure that results are comparable. The aim of this study was to compare results obtained by the participating laboratories. A mixed population of ALL cells was used as a starting point. A detailed gating strategy was set up for FC. Moreover, whether FC can reach Ig/TCR-QPCR performance in routine ALL follow-up monitoring is still questionable.

Methods. In the multicenter STIC 2006 program, MRD measurements were performed prospectively using Ig/TCR-QPCR (4 EuroMRD labs) and FC (8 labs) in 598 follow up samples from 238 patients treated for a BCR-ABL negative ALL (136 adults ALL and 102 high-risk childhood ALL); 153 had BCP-ALL and 85 had T-ALL. Both techniques were performed on the same bone marrow sample. Ig/TCR-QPCR was performed following EuroMRD guidelines. FC was performed on fresh cells. Similar antibody panels, antibody clones and gating strategies were used with 4-color (59%) or 5/7-color (61%). FC-MRD positivity was defined as a cluster of more than 10 events displaying the marker of interest in ≥ 95% of事件. Similar antibody panels, antibody clones and gating strategies were used with 4-color (59%) or 5/7-color (61%). FC-MRD positivity was defined as a cluster of more than 10 events displaying the marker of interest in ≥ 95% of events. The positive diagnosis was confirmed by RQ-PCR. SP1 protein expression was not increased in CSF-derived BCP-ALL cells (n=4) compared to BM-derived BCP-ALL cells (n=28). The absence of intracellular SP1 may partly be explained by secretion, since significantly increased soluble SP1 levels were found in ALL-positive compared to ALL-negative CSF samples. Protein expression of LEPROTL1 and other top-ranked genes is currently being evaluated on CSF- and testis-derived ALL cells. Diagnostic BM cells from four BCP-ALL patients with isolated CNS relapse were analyzed for (sub)populations of ALL cells with increased SP1 and/or LEPROTL1 expression. Clear subpopulations of ALL cells (>0.2%) with high LEPROTL1 or SP1 expression were present in 2/4 and 0/4 patients, respectively, suggesting that LEPROTL1, but not SP1 may be of prognostic value for the prediction of an isolated CNS relapse. Summary/Conclusions. EM-ALL cells and BM-derived BCP-ALL cells show distinct gene expression profiles. The presence of a small subpopulation of ALL cells with an EM signature at diagnosis may predict EM relapse. Prospective flowcytometric analysis of LEPROTL1 and other top-ranked genes in BM cells from newly diagnosed ALL patients, combined with a long-term follow-up, is ongoing to evaluate the prognostic significance of these markers. In future, the identification of proteins involved in CNS localization may contribute to the design of targeted therapies for both prophylactic treatment in patients at high risk of EM-ALL and therapeutic intervention in case of CNS relapse.

0005
THE RS564398, A POLYMORPHISM IN THE ‘ANTISENSE NON-CODING RNA IN THE INK4 LOCUS’, ANRIL, IS ASSOCIATED TO PHILADELPHIA POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) SUSCEPTIBILITY
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Introduction. Little is known about alterations of cyclin dependent kinase inhibitors p15INK4B, p16INK4A and p14ARF due to single nucleotide polymorphisms (SNPs) located within the CDKN2A/B genes and/or neighbouring loci. In order to investigate the potential involvement of such common DNA sequence variants in leukemia susceptibility, an association study was performed. Methods. 23 SNPs spanning the MTA1, CDKN2A/B and CDKN2BAS loci, as well as relative intergenic regions were genotyped in a case-control cohort made up of 149 leukemia patients, including Philadelphia positive (Ph+) ALL and acute myeloid leukemia (AML) samples, and 183 healthy controls. 6 SNPs were selected on the basis of their previous association with several diseases, such as coronary artery disease (rs2891168, rs518394, rs564398, rs10757278), type 2 diabetes mellitus (rs564398), frailty (rs2811712). The remaining 17 SNPs were selected to deepen the SNP's coverage for the examined region. Genotyping was performed using
iPLEX Gold technology and MassARRAY high-throughput DNA analysis with Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Sequenom, Inc., San Diego, CA). Results. A total of 17 SNPs, spanning the 9p genomic interval that encompasses the MTAP, CDKN2A/B and CDKN2BAS loci, were successfully genotyped and used for investigating their potential associations with the leukemic phenotypes. Five SNPs (rs1012782, rs10095719, rs40011899, rs3751252, rs3218010) with MAF <0.05 in cases and controls, as well as one SNP (rs9391609) showing >20% missing call rates, were instead excluded from the association analysis and potential population stratification affecting the control sample was ruled out as its genotypes distribution satisfies the Hardy-Weinberg equilibrium criteria. Among the 17 SNPs, rs564398, mapping to the CDKN2BAS locus (exon 2) that encodes for ANRIL antisense non-coding RNA, showed a statistically significant correlation with the ALL phenotype, with a risk ratio of 2 (95% CI, 1.20 to 3.83; p= 7.1 x 10^{-3}). Conclusion. Since a co-ordinated regulation of ANRIL and p14/ARF, p16/CDKN2A, p15/CDKN2BAS transcript has been already observed in both physiologic and pathologic conditions, we hypothesized that rs564398 association reflects a condition of high linkage disequilibrium between such polymorphism and a causative variant that is able to alter CDKN2A/B expression profiles by changing ANRIL dosage, thus leading to higher proliferative boost/s and consequent increased ALL susceptibility. Recently, the rs564398 has been demonstrated by Cun- nington M.S. et al. (2010) to alter ANRIL expression leading in turn to a deregulation of CDKN2A/B genes. Supported by European Leukemi- aNet, ALL, AIRC, HDB 2006; Ateneo RFO grants, Project of integrated programs (PIO), PRIN 2008, Programma di Ricerca Regionale - Università 2007 - 2009.

0006

GENOME-WIDE SCREENING FOR TEL-AML1 (ETV6-RUNX1) TARGET GENES

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Background and Aims. The reciprocal translocation t(12;21) (p13;q22) is the most frequent chromosomal abnormality in ALL and is associated as it occurs in approximately 25% of B-cell precursor acute lymphoblastic leukemias (ALL). The resulting chimeric transcription factor TEL-AML1 is expected to function in a dominant-negative fashion. Just as most of the genes (78%) involved in 'RNA metabolism' (e.g. gnb2l1, metap2, metap, snrk) or 'intracellular signaling pathways' (e.g. gnb2l1, metap2, snrk) were subsequently also detected in the SILAC experiments (two upregulated and eight downregulated), the clustering of affected genes directly regulated by TEL-AML1 (125 upregulated, 77 downregulated). From these direct DNA and mRNA targets, eight were subsequently also detected in the SILAC experiments (two upregulated, six downregulated): Riken cdna 90380170105 s, elk4, pdlim5, ncl, gnb2l1, hstbhk, int3, metap2. The clustering of affected biological processes regarding GO terms disclosed several distinct pathways like signal transduction, protein modification and localization as well as processes involved in regulation of transcription and RNA metabolism. As 67% of the genes corresponded to ‘regulation of transcription’ (e.g. snrp1, tal1) were found to be downregulated and 85% of those targets corresponding to ‘negative regulation of transcription’ (e.g. hlad4s, zfp3x3, foxp1) showed an upregulated expression, this observation comes up to the expectation that TEL-AML1 mainly acts as a transcriptional repressor. Just as most of the genes (78%) involved in ‘RNA metabo-
**0008**
**SEQUENCE OF MUTATIONAL EVENTS AND CLONAL ARCHITECTURE IN BCR-ABL POSITIVE ACUTE LYMPHOLBLASTIC LEUKAEMIA**

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**Background.** Intra-clonal genetic diversity is a hallmark feature of cancer and provides the substrate for sub-clonal selection, progression and therapeutic resistance. We recently demonstrated a variegated genetic architecture in the blast cells and leukaemia-propagating ("stem") cells of childhood ETv6-RUNX1-positive acute lymphoblastic leukemia (ALL). Single cell analysis with multi-colour FISH probes (Anderson et al., Nature, 469: 356-361, 2011). Aims. In the present study, we aimed to determine whether this genetic diversity also applies to high risk ALL with BCR-ABL fusion. Methods. We used multiplexed FISH analysis of single cells using probes labelled with five distinct fluorochromes. We screened diagnostic blast cells samples from B-BCR-ABL-positive cases for deletion of IKAROS (IKZF1), CDKN2A/p16 and PAX5. The application of multiple FISH probes allowed us to score all cells (200 per patient sample) simultaneously for the BCR-ABL fusion (and multiple copies of the fusion), and one or two copy deletions of IKZF1, PAX5 and CDKN2A. Results. The genetic classification of individual cells allowed a designation of sub-clones and the assembly of putative ancestral trees. Four of the eight cases screened had concurrent IKZF1, PAX5 and CDKN2A deletions and in two of these we could distinguish the order acquisition of the deletions. In one case the IKZF1 and CDKN2A/PAX5 deletions arose independently in different sub-clones; i.e. IKZF1 was deleted first in one subclone and CDKN2A/PAX5 first in another subclone. In the second case the CDKN2A deletion arose subclonally to both the Ikaros and PAX5 deletions. We also studied one patient with matched diagnosis and relapse samples available. Comparison of SNP array data at diagnosis versus relapse revealed that different (or reiterated) genomic regions involved in the two distinctive IKZF1 deletions (diagnosis versus relapse). FISH analysis then allowed us to identify clonal substructure at diagnosis, including a minor sub-clone (1%) with the genotype of the dominant clone seen in relapse. Summary/Conclusions. These results indicate that the sub-clonal architecture in BCR-ABL positive ALL is genetically diverse. The common CNA are acquired in no preferential order, and CNA involving the same gene can arise independently in different sub-clones. An important prediction derived from this pattern of sub-clonal architecture is that the leukaemia propagating cells in BCR-ABL ALL should themselves be genetically diverse. We plan to perform in vivo transplantation experiments in NOD/SCID/γc-/- mice with leukaemic cells from patients for whom we find clonal heterogeneity. Regenerated leukaemias will be re-transplanted and the subsequent leukaemias analysed by both SNP arrays and multi-colour FISH to compare genetic diversity of stem cell output and the subsequent leukaemias. Aims. To establish the role of components of the PI3K/AKT/mTOR signaling pathway in different subtypes of ALL to overcome resistance to TKI. Methods. The effects of selective inhibitors of PI3K (NVP-BKM120) and mTORC1 (RAD001) and of dual PI3K/mTORC1/C2 inhibitors (NVP-BEZ235 & NVP-BGT226) on long-term cultured human ALL cells (n=6) B-precursor ALL cells were analysed. All inhibitors were kindly provided by Novartis, Basel, Switzerland. Cell proliferation and apoptosis were measured by XTT-assays and FACS analysis using annexin V/propidium iodide. Phosphorylation of the proteins 4E-BP1 (Thr37/46) & S6 Ribosomal Protein (Ser235/236) downstream of PI3K/mTORC1/C2 inhibitors (NVP-BEZ235 & NVP-BGT226) were more potent in inhibition of cell proliferation and induction of apoptosis in Ph+ and Ph neg. ALL than the selective PI3K and mTORC1 inhibitors alone. The anti-proliferative and pro-apoptotic effects of these inhibitors were independent of bcr-abl and partial resistance to 1st and 2nd generation TKI. Comparison of the effect of selective PI3K and mTOR inhibitors on mTOR signaling revealed differential regulation of S6 and 4E-BP1. Whereas selective inhibition of PI3K and mTORC1 by BKM120 and RAD001, respectively, resulted in dephosphorylation only of the S6 protein, combined inhibition of PI3K and mTORC1 was associated primarily with a decrease of S6 phosphorylation and only minor dephosphorylation of 4E-BP1. Conversely, exposure to the dual PI3K/mTORC1/C2 inhibitors resulted in nearly complete dephosphorylation of both S6 and 4E-BP1. Summary/Conclusions. Our observation that compounds inhibiting PI3K/mTORC1/C2 have significantly greater anti-proliferative and pro-apoptotic effects than selective inhibition of PI3K and mTORC1 support a functional role of mTORC2 in survival and growth of B-precursor ALL cells. Our data indicate that mTORC2 contributes substantially to regulation of the downstream target 4E-BP1 by mTORC1 in ALL. Combined targeting of these complex may provide a novel therapeutic approach for both Ph+ ALL resistant to ABL TKI and Ph neg. ALL.

**0009**
**MTORC2 PLAYS A DISTINCT ROLE IN MEDIATING ANTI-PROLIFERATIVE AND PRO-APOTOTIC EFFECTS OF INHIBITORS OF THE PI3K/AKT/MTOR CASCADE IN LONG-TERM CULTURED PRIMARY ACUTE LYMPHOLBLASTIC LEUKAEMIA (ALL) CELLS**

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**Background.** The PI3K/AKT/mTOR pathway is considered as a downstream signaling pathway of the bcr abl oncogene that is the hallmark of Philadelphia chromosome positive (Ph+) acute lymphoblastic leukaemia (Ph+ ALL) and of CML. Activation of the PI3K signaling pathway has been suggested to play a role in resistance of Ph+ ALL to tyrosine kinase inhibitors (TKI) that target BCR ABL kinase activity. Its role in Ph neg. ALL has not been clearly established. mTOR is a kinase and catalytic subunit of the two complexes mTORC1 and mTORC2. Whereas mTORC1 predominantly controls cell growth, mTORC2 appears to mediate cell proliferation and cell survival. mTORC1 phosphorylates the translational regulators 4E-BP1 and S6K1 and 4E-BP1. The central role of mTORC1 regulation in oncogenic PI3K signaling provides strong rationale for targeting mTORC1 in cancer, but mTORC1-dependent negative feedback loops mitigate the effectiveness. mTORC2

**0010**
**PRECLINICAL ACTIVITY OF LBH589 ALONE OR IN COMBINATION WITH CHEMOTHERAPY IN A XENOGRAFIC MOUSE MODEL OF HUMAN ACUTE LYMPHOLBLASTIC LEUKAEMIA**

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**Background.** Histone deacetylases (HDACs) have been identified as therapeutic targets due to their regulatory function in chromatin structure and organization. Here we analyzed the therapeutic effect of LBH589, a class I-II HDAC inhibitor, in acute lymphoblastic leukaemia (ALL). In vitro, LBH589 induced dose-dependent antiproliferative and apoptotic effects, which were associated with increased H3 and H4 histone acetylation. Intravenous (i.v.) administration of LBH589 in immunodeﬁcient BALB/c-RAG2-/- mice in which a human-derived T and B-ALL cell lines were injected induced a signiﬁcant reduction in tumor growth. Using primary ALL cells, a xenograft model of human leukemia in BALB/c-RAG2-/- mice was established, allowing continuous passages of transplanted cells to several mouse generations. Treatment of mice engrafted with T or B-ALL cells with LBH589 induced an in vivo increase in the acetylation of H3 and H4, which was accompanied with prolonged survival of LBH589-treated mice in comparison with those receiving Vincristine and Dexametason. Notably, the therapeutic efficacy of LBH589 was significantly enhanced in combination with Vincristine and Dexametason. Our results demonstrate the therapeutic activity of LBH589 in combination with standard chemotherapy in pre-clinical models of ALL and suggest that this combination may be of clinical value in the treatment of patients with ALL.
THE INSULIN RECEPTOR SUBSTRATE 4 GENE IS MUTATED IN PAEDIATRIC T-CELL ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background. The cytogenetic hallmark of T-cell acute lymphoblastic leukaemia (T-ALL) is the presence of rearrangements involving the T-cell receptor (TCR) loci, mainly TRA6/TRD2 at 14q11 and TRB2 at 7q34, that dysregulate a variety of genes with oncogenic potential. Several genes involved in TCR translocations have also been shown to be targeted by alternative mechanisms. NOTCH1 is the most pertinent example of this. This gene was initially shown to be rearranged by the rare t(7;9)(q34;q34), but is now known to harbour activating mutations in the majority of T-ALL cases. Thus, mutation analysis of an infrequent TCR target may well reveal that it plays a greater role in T-ALL development than surmised based on the incidence of the translocation alone. Aims. We recently reported a novel translocation - t(X;7)(q22;q34) - in a paediatric T-ALL and showed that it results in overexpression of the insulin receptor substrate 4 (IRS4) gene. We speculated that this gene could be mutated in additional cases, akin to what has been reported for NOTCH1. In the present study, we have therefore sequenced IRS4 in paediatric T-ALL samples. Methods. The patients (n = 21) with T-ALL represents 78% (21/27) of all T-ALL (<15 years) diagnosed in Lund and Linköping 1990-2007 and comprises all cases from which DNA from diagnostic samples was available. The IRS4 gene at Xq22.3 consists of only one exon (3,881 bp; NM_003604.2), which was amplified by nested extra long polymerase chain reaction (Applied Biosystems, Foster City, CA, USA). Sequencing of the antisense strand of IRS4 was carried out by the GATC sequencing service (Konstanz, Germany). The sequences were analysed using the Mutation Surveyor software (Softgenetics LLC, State College, PA, USA). Results. The entire IRS4 gene was successfully sequenced in all 21 cases, of which 19 displayed a sequence matching the reference (NM_003604.2). Two cases had sequences that deviated from the reference but which have not been reported as polymorphisms, suggesting that they were mutations. Case 3 displayed an in-frame 594 bp deletion - c.105_698del (NM_003604.2); p.24_221del (NP_003595.1) indicated in figure 1A with a dotted line. Unfortunately, no remission sample was available to ascertain whether this deletion was acquired or constitutional. The found deletion results in the removal of the pleckstrin homology domain represented by a black box in figure 1B whereas the blue box indicates the segment reported as a copy number variation in Yoruba Nigerians (http://projects.tcag.ca/variation/). The pleckstrin homology domain is functionally important and well conserved among the IRS proteins. Case 8 harbouring a missense mutation - c.670C>A (NM_003604.2); p.Pro215Thr (NP_003595.1), the position is marked by a red asterisk in figure 1B and 1C. The mutation was not found in either a remission or a relapse sample. Threonine residues have occasionally been reported to be phosphorylated in IRS proteins resulting in inhibition of the IRS activity. Summary. We have for the first time identified IRS4 mutations in T-ALL. Whether such mutations confer a specific biological or clinical impact remain to be investigated in larger patient cohorts.

DISTINCT ACCESSIBILITY OF TLX1 AND LMO2 BREAKPOINT SITES MODULATES INVOLVEMENT IN T-CELL RECEPTOR (TCR)-ASSOCIATED TRANSLATION FORMATION

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Background. T-cell receptor (TCR) translocations are a genetic hallmark of T-cell acute lymphoblastic leukaemia (T-ALL). Two well known TCR translocation partners, LMO2 and TLX1, have shown to be recurrently involved in these translocations, and are therefore considered to be more prone to the induction of DNA double-strand breaks (DSB) at time of V(D)J recombination. LMO2 and TLX1 are respectively regarded as "Type 1" and "Type 2" translocation models. LMO2 translocations mostly involve RAG mediated DSB inductions due to the presence of a cryptic recombination signal sequence (cRSS), while the cause of TLX1 DSB induction is unknown. We hypothesize that chromatin modulation during thymocyte development renders DNA vulnerable to DSB induction. This vulnerability is thought to facilitate both "Type 1" and "Type 2" translocations during V(D)J recombination. Aims. We aimed to determine the accessibility of LMO2 and TLX1 at breakpoint sites (BPS) during thymocyte development. We did this in order to assess whether there is a correlation between accessibility of BPS during thymocyte development and the involvement of these sites in translocations. Methods. We isolated DNA of thymocyte subsets by FAIRE (Formaldehyde Assisted Isolation of Regulatory Elements) to quantitatively determine nucleosome occupancy throughout thymocyte development. Furthermore, we isolated thymocyte DNA to determine the methylation status of these BPS by means of bisulfite sequencing. Obtained data were correlated to the thymocyte developmental stages at which TCR loci are rearranged and to the occurrence of involvement of the BPS in translocations. Results. Our findings show some level of nucleosome depletion on oncogene BPS, with no clear increase in accessibility at a particular thymocyte developmental stage. Nucleosome enrichment levels correlate to the methylation status of the BPS within the LMO2 locus. However, the LMO2 translocation hotspot, at which a functional cRSS is located, is hardly nucleosome depleted and is mostly hypermethylated. In contrast, all TLX1 BPS showed mostly hypomethylated status. In the TLX1 translocation hotspot showing high levels of nucleosome depletion. Conclusions. Nucleosome depletion combined with a hypomethylated status seems to renders BPS within the TLX1 translocation hotspot more prone to involvement in TCR-associated translocations. The LMO2 translocation hotspot is hardly accessible during thymocyte development, perhaps an indication that another type of accessibility is needed for RAG targeting. No clear chromatin modulation at the LMO2 and TLX1 locus is seen during thymocyte development. This implies that other conditions are imperative in driving the occurrence of T-ALL related translocations in specific thymocyte stages.

IS IMATINIB-RESISTANCE IN BCR-ABL1-POSITIVE LEUKEMIA DUE TO LOSS OF PTEN AND/OR ACTIVATING PIK3CA MUTATIONS?

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Background. The BCR-ABL1 translocation occurs in 95% of cases with chronic myeloid leukaemia (CML) and in 25% of cases with acute lymphoblastic leukaemia (ALL). Tyrosine kinase inhibitors (TKI) - routinely administered in CML - are not equally effective for treating ALL. Causes for imatinib-resistance are: (i) the development of clones carrying mutations in the kinase domain of BCR-ABL1, (ii) low intracellular drug levels caused by disordered influx and efflux transport, (iii) overexpression of BCR-ABL1, and (iv) activation of alternative signaling pathways by oncogenic enzymes like SRC kinases. Many studies performed to elucidate imatinib-resistance use of cells ec-topically expressing BCR-ABL1 or of cell lines which gained resistance after chronic exposure to rising drug concentrations. Aims. To find models for TKI-resistance, we analyzed nineteen BCR-ABL1-positive cell lines and found that five cell lines (KL-22, MHH-TALL1, NALM-1, SD-1, SUP-B15) were resistant to imatinib and nilotinib. We set out to investigate whether these cell lines displayed the known molecular causes for TKI-resistance. Methods. TKI-resistance was determined by (3H)-thymidine uptake and annexin-V binding assays. Activity of signal transduction pathways was tested by Western blot analysis.
using antibodies against phosphorylated BCR-ABL1 targets. Mutational analysis of the BCR-ABL1 kinase domain, of PIK3CA and of PTEN were performed by DNA sequencing. Results. None of the resistant cell lines carried mutations in the kinase domain of BCR-ABL1 or other molecular aberrations previously mentioned in the context of TKI-resistance. The cell lines dephosphorylate the BCR-ABL1 downstream targets ERK1/2 and STAT5 after treatment with imatinib, while PIK3CA mutation in the resistant cell lines promotes the AKT1 inhibitors promoted dephosphorylation of the mTOR target RPS6 and induced apoptosis. Cell line KCL-22 carried a PI3Kalpha E545G mutation, a site critical for the constitutive activation of the enzyme, indicating that mutations in the PI3K itself may be responsible for imatinib-resistance. However, loss of tumor suppressor genes can activate oncogenic pathways. Accordingly, the T-ALL cell line MHH-TALL1 was found to carry a missense mutation in the PI3K-counteracting phosphatase PTE1 leading to truncation of the protein after amino acid 241. Quantitative genotypic PCR analysis revealed that the second PTEN allele was deleted in this cell line. Summary/Conclusions. 5/19 BCR-ABL1-positive cell lines tested were TKI-resistant. All imatinib-resistant cell lines were responsive to TKI regarding the BCR-ABL1-downstream targets STAT5 and ERK1/2, but resistant with respect to the PI3K/AKT1 pathway. One of the resistant cell lines (KCL-22) carried a PI3Kalpha E545G mutation, a second (MHH-TALL1) did not express the full-length version of the PI3K-counteracting tumor suppressor gene PTEN. Either aberration might be responsible for the constitutive PI3K activity, and thus also be responsible for TKI-resistance. Our results highlight the importance of the PI3K in imatinib-resistance.

0014 FREQUENT METHYLATION AND DECREASED EXPRESSION OF THE RIZ1 GENE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA OF T CELL PHENOTYPE

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Background. Retinoblastoma protein-interacting zinc finger gene, RIZ1 has characteristics of a negative regulator of tumorigenesis and is considered to be a tumor suppressor gene. The RIZ1 gene is inactivated in various tumors by hypermethylation, and epigenetic silencing is the common process behind RIZ1 inactivation. In hematological malignancies, RIZ1 expression was found to be significantly decreased in leukemia cell lines, primary samples from acute myeloid leukemia and chronic myelogenous leukemia during the transformation from chronic phase to blastic crisis. However, the role of the RIZ1 gene has not been well examined in adult acute lymphoblastic leukemia (ALL). Aims. The aims of this study are to assess the RIZ1 expression and altered methylation status in adult ALL and to determine the association between these features and clinical characteristics of the patients. Methods. We examined expression of the RIZ1 gene by quantitative real-time reverse transcription-polymerase chain reaction (PCR) analysis and performed methylation-specific PCR on the RIZ1 gene in newly diagnosed 73 adult ALL patients (62 B-ALL and 11 T-ALL). Normal bone marrow cells (n=10), normal lymphocytes (n=3) and lymphoid cell lines (n=4) were also examined. Characteristics of the patients including age, sex, phenotype of ALL, WBC counts, LDH at the diagnosis, karyotype and response to induction chemotherapies were also investigated. MOLT-4 cells that have the RIZ1 methylation were treated with DNA methyltransferase inhibitor, 5-Aza-dC. Results. RIZ1 expressions of 67 ALL (mean 1.043) were decreased compared with those of normal bone marrow mononuclear cells (mean 1.47) (P < 0.05). The RIZ1 expressions in the T-ALL patients (mean 0.606) were lower than those in the B-ALL patients (mean 1.145) (P = 0.045), although the expressions in normal T-cells (mean 6.933) were higher than those in normal B-cells (mean 3.229). The expression was not associated with other clinical characteristics. Methylation of the RIZ1 promoter was detected in 11 of the 71 patients (15.5%) while it was absent in healthy controls. The RIZ1 methylation was accompanied with decrease of RIZ1 mRNA levels among 9 of the 11 methylation-positive patients (81.8%), although there was no difference in the expressions between methylation-positive (mean 0.818) and -negative patients (mean 1.114) (P = 0.151). RIZ1 methylation was more frequent in T-ALL (63.6%) than in B-ALL (5.0%) (P < 0.0001), and more frequent in Philadelphia chromosome (Ph)-negative ALL (23.3%) than in Ph-positive ALL (6.7%) (P = 0.027). We found no correlations between the methylation status and other clinical characteristics. 5-Aza-dC treatment of MOLT-4 cells induced demethylation of the RIZ1 promoter and restored expression of cell proliferation and viability in a dose- and time-dependent manner. Restoration of the RIZ1 expression was induced in the cells treated with a high concentration of 5-Aza-dC. Summary/Conclusions. RIZ1 expression is decreased in adult ALL. Decreased expression and methylation of the RIZ1 gene are frequent in T-ALL. 5-Aza-dC treatment of MOLT-4 cells induced demethylation of the RIZ1 promoter and restoration of the RIZ1 expression. These results suggest that RIZ1 is inactivated in adult ALL and this inactivation is associated with methylation in T cell phenotype.

0015 GERMLINE GENOMIC VARIATIONS AT IKZF1, ARID5B, AND CEBPE GENES FOR THE PREDICTION OF DEVELOPING CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREA

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Background. Recent western studies have shown the implication of the germline genomic variations in IKZF1 gene at 7p12.2, ARID5B gene at 10q21.2, and CEBPE gene at 14q11.2 on the risk of childhood acute lymphoblastic leukemia (ALL), the most significant association was observed in the single nucleotide polymorphism (SNP) rs4132601 which located at 3’ region of the IKZF1. IKZF1 plays an important role in lymphocyte differentiation, proliferation and function, ARID5B in embryogenesis and growth regulation, and CEBPE in regulation of myelopoiesis. Genetic variants in these genes are therefore considered to be involved in transcriptional regulation and differentiation of B cell progenitors. However, there have been no reports on the role of germline variations in leukemogenesis of childhood ALL in Asian countries. Aims. The aim of this study is to show the impact of these genetic variants on childhood ALL in Korea. Methods. To examine the association between genetic variations (IKZF1 rs4132601, ARID5B rs7089424, and CEBPE rs2239633) and the risk of childhood ALL, we analyzed 228 children with ALL and 506 healthy individuals in Korea. Results. In ARID5B rs7089424, TG and GG genotypes were significantly associated with a risk for ALL (odds ratio [OR], 1.63; 95% confidence interval [CI], 1.07-2.48; P=0.02) for TG genotype, OR, 2.69; 95% CI, 1.42-5.07; P=0.002 for GG genotype). The allele incidence of ARID5B rs7089424 was also significantly associated with a risk for ALL (OR, 1.66; 95% CI, 1.24-2.22; P<0.0006). The allele incidence of CEBPE rs2239633 TT genotype showed a significant association with a decreased risk for ALL (OR, 0.54; 95% CI, 0.33-0.90; P=0.02) for TT genotype). The allele incidence of CEBPE rs2239633 was also associated with a decreased risk for ALL (OR, 0.77; 95% CI, 0.61-0.97; P=0.02). There was no significant association between IKZF1 rs4132601 polymorphism and a risk for ALL in this study. Conclusions. These results suggest that germline variations of ARID5B and CEBPE may play an important role in the risk for childhood ALL in Korea, compared with findings from western countries showing a significant relation between IKZF1 and childhood ALL. Several factors should be considered to explain a discrepancy between our results and the previous studies, which include different genotype frequencies in polymorphisms and varied susceptibility to ALL in different ethnic groups. Further studies incorporating larger number of cases and analyzing other SNPs or other Asian countries are warranted in childhood ALL.
PARALLEL SAMPLING OF BONE MARROW AND PERIPHERAL BLOOD IS ADVISED FOR THE MOLECULAR EVALUATION OF MINIMAL RESIDUAL DISEASE IN BOTH B AND T LINEAGE ADULT ACUTE LYMPHOBlastic LEUKEMIA

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Background. An accurate detection of minimal residual disease has become a crucial tool to evaluate prognosis and therapeutic strategies both in childhood and adult patients with acute lymphoblastic leukemia (ALL). The need of repeated (BM) sampling during the clinical course can be problematic for many patients. Preliminary results obtained in children showed that BM sampling can not be replaced by PB in B precursor ALL, but could be considered for T-ALL. Here we report our comparative analysis performed in a large prospective study performed in adult B and T ALL. Material and Methods. One hundred and seven adult patients (73 B-precursor ALL and 34 T lineage ALL) enrolled 2 consecutive clinical trials launched by NILG, were monitored for molecular MRD in BM and PB. Two informative Ig or TCR derived molecular probes (with a sensitivity ranging from 10−3 to 10−5) were used in 77 patients (51 B-precursor and 26 T-ALL) while in only one probe was available for 30 (22 B-precursor and 8 T-ALL). A paired BM/PB analysis was conducted on 721 paired samples (500 from B and 221 from T-ALL) by real-time quantitative PCR (RT-qPCR). Results. In T-ALL 132 out of 221 paired samples obtained during follow-up proved negative both in the BM and PB whereas a positive MRD was found in at least one sample of the remaining 89 paired analyses. Among these latter samples, a positive MRD was detected in both BM and PB with comparable levels of residual disease in 47 paired samples (53%), while the amount of detectable disease was remarkable different (from 1 to 2 log) in 23 pairs (26%). In 8 paired samples (9%) MRD proved positive only in the BM while in 11 (12%) only in the PB. In B precursor ALL 200 out of 500 paired samples showed a measurable MRD level in the BM and/or PB (figure 1 panel A). In 46 paired samples (23%) the amount of MRD was similar in BM and PB while in 72 (36%) MRD was significantly higher (up to 3 log difference) in the BM compared to PB. In only one case (0.5%) a higher MRD level was detected in PB. In 66 paired samples (33%) MRD was detectable only in the BM and in several of these cases the amount of residual disease could be as high as 10-2. In 16 pairs (8%) only the PB proved positive but at very low levels (usually ≤ 10-4) (figure 1 panel B). Conclusion. Although MRD detection on BM samples is more sensitive compared to PB, in both B and T lineage ALL. However, in some T-ALL cases, the PB may provide discordant and informative results. All in all, our results suggest that either in B precursor ALL as well as in T-ALL, an MRD evaluation must be always performed on BM but preferably, PB samples should be tested in parallel.
B-CELL ACUTE LYMPHOBlastic LEUKEMIA NEGATIVE FOR CD38: A SUBSET OF PATIENTS WITH DISTINCT BIOLOGICAL AND PROGNOSTIC FEATURES?

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Background. It is known that CD38 expression is associated with poor prognosis in patients with chronic lymphocytic leukaemia; however, its significance is unclear in other lymphoid malignancies. Although some authors have suggested that the negativity of the blasts for CD38 is correlated with a worse prognosis in patients with B-cell acute lymphoblastic leukaemia (B-ALL), it has yet to be clarified. Aims. The purpose of this study was to evaluate the main characteristics of patients with B-ALL according to CD38 expression. Methods. We analyzed the features at diagnosis and the evolution of 44 patients with B-ALL treated at two hospitals in our community over the past ten years. The group consisted of 17 pediatric patients (age below 18 years) and 27 adults, with a median age of 26.5 years (range 2-75) and women 54%. Recorded data included morphology, laboratory findings, immunophenotype, cytogenetics and FISH, clinical features, treatment approach, follow-up and survival. Statistical analysis was performed using SPSS software, and relations between variables were studied using the Fischer exact test (categorical) and the Mann Whitney U test (continuous). Results. In 33 patients lymphoblasts were positive for CD38, while eleven did not express this cell surface marker. When compared with CD38 positive group the blasts of patients negative for CD38 were more frequently CD34, KOR-SA and CD13 positive (p=0.05, p=0.013 and p=0.049, respectively), and CD20 and CD22 negative (p=0.014 and p=0.05). Of note, 86% of patients lacking CD38 presented with BCR-ABL1 fusion gene, and only 14% in the CD38 positive group (p=0.001). Although overall survivals were similar, mortality tended to be higher among the CD38 negative B-ALL cases compared with CD38 positive B-ALL group, the blasts of patients negative for CD38 had a higher proportion of the death promoter to death repressor gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels of p53 target genes, it had no effect on p53 gene expression levels
ticularly relevant to T-ALL where up to 40% of patients have no detectable cytogenetic abnormality. In recent years whole genome microarray analysis has improved our understanding of T-ALL pathogenesis with the identification of multiple novel copy number alterations (CNA). We applied this technology to testing the hypothesis that AYA ALL has a unique genetic profile. Aims. Our aim was to perform whole genome microarray analysis in a cohort of AYA T-ALL patients to determine the frequency of known genetic aberrations in our cohort compared to previous pediatric studies and to determine the presence of any novel copy number alterations (defined as recurrent CNA present in two or more samples in our cohort and not described in previous series of T-ALL). Methods. Diagnostic DNA from 25 AYA T-ALL patients (age 10 - 25 years) was subjected to copy number analysis using the Affymetrix SNP Array 6.0 platform, and CNAG 3.3.0.0 software for data analysis. Each CNA was classified as a passenger or driver: CNAs were classified as passengers if they were a known variant present in healthy controls or if they had no known/postulated role in oncogenesis. CNAs were classified as drivers if they were recurrent changes or if they had a known/postulated role (e.g known oncogene from another malignancy) in leukaemogenesis. Results. The mean number of drivers was 3.8 and the median was 3 (range 1 - 9). Frequency of known CNA in AYA T-ALL in our series was similar to paediatric studies with deletion of p16 again playing a pivotal role. Frequencies of deletion of 1p36, 1p31.3, 6q14-15, 14q32, 14q32.3 (leading to upregulation of the oncogene p53), 1q21, 2q22.1 (POT1, HOXA and HOXB), 1q32.1, 2q32.1 (2q32 and HOXA respectively) and deletion/gain of LEF1, MYB and PTEN were similar to previous paediatric references (see Table 1). A number of individual samples had copy number changes in genes usually associated with other haematological malignancies including loss of ARHGAP26 (MMALL) and gain of 11p13 (CLL). Potential novel areas of recurrent CNA identified included gain of 1p22.3 and deletion of 3q12.2. Conclusions. Copy number ‘drivers’ in our AYA T-ALL cohort were surprisingly similar to the paediatric population. However, the hypothesis that AYA T-ALL represents an independent group at a molecular genetic level cannot be rejected without expansion of this approach to sequence based mutations and cryptic translocations (undetectable by microarray). Gain of 1p22.3 in 12% samples implicated CLCA4, a chloride channel as warranting further study, and deletion of 3q12.2 in 8% of the samples is of interest as this corresponds to a partial deletion of the TFG gene previously implicated in oncogenic rearrangements in anaplastic lymphoma.

Table 1.

<table>
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<th>Genotype</th>
<th>Allele A</th>
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<tr>
<td>3q12.2</td>
<td>G</td>
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<td>1p22.3</td>
<td>C</td>
<td>T</td>
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<td>HOXA</td>
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**Background.** Lymphoblastic T-cell lymphoma (T-LBL) and T-cell acute lymphoblastic leukemia (T-ALL) are lymphoid neoplasms characterized by the proliferation of malignant T lymphoblasts arrested at early stages of maturation. They differ by the extent of bone marrow involvement, which is <25% in T-LBL. Since they share several biological characteristics, including cytology and immunophenotype they tend to both be treated on T-ALL protocols. Relapsed patients with either disease do badly, but the time to relapse tends to be shorter in T-LBL than in T-ALL as sites of relapse differ. AYA patients have superior prognosis compared to childhood T-ALL. In a homogenous cohort of 47 pediatric T-LBL, only 1 (2.1%) patient had a clinical event: 1 with NOTCH1 mutation without FLASH deletion, 4 with FLASH deletion without N/Fwt and 4 with neither deletion. NOTCH1 and/or FBXW7 mutations (N/Fwt) were found in 24 of 47 T-LBL, which was a group with superior prognosis considering the EFS (p=0.01). NOTCH1 mutations were found in 45% (21/47) and FBXW7 mutations in 15% (6/45) of the patients with FL deficiencies (FLASH monosomy, 1q22, HOXA, 1q21). Similar to the previously published incidence of del6q (19%), we observed no biallelic FLASH deletions. The majority of patients with FLASH deletion were not mutated for NOTCH1 or FBXW7 (N/Fwt) but this was not statistically significant. To date, 9 patients with leukemia from this cohort had a clinical event: 1 with NOTCH1 mutation without FLASH deletion, 4 with FLASH deletion without N/Fwt and 4 with neither FLASH deletion nor N/Fwt. Conclusion. FLASH genetic dosage represent a simple alternative to LOH or CGH to identify T-LBL with del6q. FLASH deletions do not appear to accentuate the bad prognosis of N/Fwt-T-LBL. Given that FLASH deletion is found preferentially in N/Fwt cases it is possible that its poor prognosis reflects the absence of N/Fwt, rather than being directly related to the del6q.

**0023**

**IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA THE RISK EFFECT OF NQO1 C609T POLYMORPHISM AND THE PROTECTIVE EFFECTIVE OF MTHFR A1298C POLYMORPHISM NEUTRALIZE EACH OTHER**

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**Background.** Xenobiotic-metabolizing enzymes constitute an important line of defense against a variety of carcinogens. Many are polymorphic, constituting the basis for the wide inter-individual variation in metabolic capacity and possibly a source of variation in the susceptibility to chemical-induced carcinogenesis. Aims. The aim of this study was to determine the existence of any association between the genetic polymorphisms of NQO1 C609T & MTHFR C677T & A1298C and altered risk for pediatric ALL. Methods. A total of 91 patients and 311 controls were genotyped by means PCR-RFLP-based assays. Mutated alleles comprising NQO1 C609T & MTHFR C677T & A1298C were analyzed along with the wild-type alleles. Results. The frequency of NQO1 609CT heterozygous genotype was 42.6% among patients compared to 37 (30.2%) among controls; the difference was found to be statistically significant (P = 0.003, O.R=2.214 & 95% C.I = 1.343 - 3.649). The frequency of the NQO1 609TT homozygous genotype was 8 (6.6 %) among patients compared to 13 (4.5%) among controls; the difference was found to be statistically insignificant (P = 0.715, O.R=1.04, 95% C.I = 1.099 - 1.874). The frequency of the MTHFR A1298C heterozygous genotype was significant higher in the control group 140 (45%) however in ALL group 22 (25%) (P = 0.001, OR=0.382 and 95% C.I=0.222 - 0.465). the MTHFR A1298C heterozygous genotype was found to be statistically significant (P = 0.003, OR=2.214 & 95% C.I = 1.343 - 3.649). The frequency of the NQO1 609TT homozygous genotype was 8 (6.6 %) among patients compared to 37 (30.2%) among controls; the difference was found to be statistically insignificant (P = 0.280). the frequency of combined genotype MTHFR A1298C heterozygous and NQO1 609CT homozygous (ACT1) was 2 (2.4%) among patients compared to 7 (2.5%) among controls; the 2 figures are comparable (P = 0.969) (Table 50). The protective effect of MTHFR A1298C allele and the risk effect of NQO1 C609T neutralize each other. Summary
and Conclusion. on a separate analysis the NQO1 609Ct&TT associated with increased risk of pediatric ALL while MTHFR 1298AC associated with protective effect. On combined analysis, both effete neutralize each other.

0024
MUTATION OF THE RECEPTOR TYROSINE PHOSPHATASE PTPRC (CD45) IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Deregulated tyrosine kinase signaling is an important factor that contributes to the enhanced proliferation and survival of leukemic cells. In T-cell acute lymphoblastic leukemia (T-ALL), activation of tyrosine kinases (ABL1, JAK1, LCK, FLT3) has been reported in approximately 20% of patients. Additionally, our group recently reported deletions of the tyrosine phosphatase PTPN2 in 6% of T-ALL cases. Loss of PTPN2 was shown to potentiate oncogenic kinases as well as influence response to kinase inhibitor treatment.

Aims. It can be expected that additional tyrosine kinases and phosphatases remain to be discovered that play a prominent role in the pathogenesis of T-ALL. To identify novel therapeutic tyrosine kinase targets in T-ALL, we screened a panel of 10 T-ALL cell lines with an siRNA library targeting the human tyrosine kinome.

Results. Our siRNA screen successfully identified known critical tyrosine kinases in three control leukemia cell lines (NUP214-ABL1 in ALL-SIL cells, FIP1L1-PDGFRA in EOL-1 cells, and LCK in HSB-2 cells), establishing it as a reliable tool for target identification. Furthermore, our screen identified several critical tyrosine kinases in other T-ALL cell lines, including LCK in two cell lines (NTRK2, and JAK1 in three cell lines). We identified and confirmed JAK1 to be an essential kinase for the proliferation of DND41 cells. The JAK1 gene itself was not mutated in DND41, but we identified a heterozygous nonsense mutation in the PTPRC gene. PTPRC encodes the tyrosine phosphatase CD45, a known negative regulator of JAK kinases. In functional assays in T-cell lines, siRNA mediated knockdown of CD45 indeed caused increased JAK/STAT signaling downstream of cytokine receptors. Sequence analysis of PTPRC subsequently identified missense and nonsense mutations in 3 of 15 T-ALL cell lines and 5 of 65 primary T-ALL cases at diagnosis. Conclusion. Our data demonstrate that siRNA screens can identify tyrosine kinases that are essential for the proliferation of T-ALL cells. Based on these results, we identified loss-of-function mutations in the receptor tyrosine phosphatase CD45 (PTPRC). Our data provide genetic and functional evidence for a tumor suppressor function for CD45.

0025
C-JUN PROMOTES BCR-ABL INDUCED LYMPHOID LEUKEMIA BY INHIBITING METHYLATION OF THE 5′ REGION OF CDK6

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Background. The transcription factor c-JUN and its upstream kinase JNK1 have been implicated in BCR-ABL induced leukemogenesis. JNK1 has been shown to regulate BCL2 expression thereby altering leukemogenesis, but the impact of c-JUN remained unclear. Aims. In this study we show that JNK1 and c-JUN promote leukemogenesis via separate pathways, since lack of c-JUN impairs proliferation of p185BCR-ABL transformed cells without affecting viability. Methods/Results. The decreased proliferation of c-Jun cells is associated with the loss of cyclin dependent kinase 6 (CDK6) expression. In c-Jun cells CDK6 expression becomes down-regulated upon BCR-ABL induced transformation which correlates with CpG island methylation within the 5′ region of CDK6. We verified the impact of CdK6 deficiency by using CdK6-/- mice that developed BCR-ABL induced B-lymphoid leukemia with significantly increased latency and an attenuated disease phenotype. In addition we show that re-expression of CDK6 in BCR-ABL transformed c-Jun cells reconstitutes proliferation and tumor formation in Nu/Nu mice. Summary. In summary, our study reveals a novel function for the transcription factor c-JUN and its upstream kinase JNK1 have been implicated in BCR-ABL induced leukemogenesis. JNK1 has been shown to regulate BCL2 expression thereby altering leukemogenesis, but the impact of c-JUN remained unclear. Aims. In this study we show that JNK1 and c-JUN promote leukemogenesis via separate pathways, since lack of c-JUN impairs proliferation of p185BCR-ABL transformed cells without affecting viability.
Acute myeloid leukemia - Biology 1

0026

SMALL SOMATIC MUTATIONS IN ACUTE PROMYELOCYTIC LEUKEMIA (APL) IDENTIFIED BY EXOME SEQUENCING

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The t(15;17) translocation which results in the PML/RARA fusion is the disease defining lesion in nearly all cases of acute promyelocytic leukemia (APL). Despite the importance of the PML/RARA fusion for the pathogenesis of APL it is most likely not sufficient to cause leukemia alone. For example, collaborating mutations affecting the FLT3 receptor tyrosine kinase are found in about 20-30% of APL patients. To screen systematically for additional mutations, we performed whole exome sequencing of 3 APL patients. Thereby, we generated at least 5 Gbp of exome sequence for each of the APL samples and for each of the corresponding normal samples. This allowed us to cover at least 80% of RefSeq coding exon positions with a minimum read depth of 10 and at least 75% of RefSeq coding exon positions with minimum read depth of 20. By comparing the APL exome sequence with the remission exome sequence, we screened for small APL-specific genetic variants. We were able to confirm 3 of 8 somatic mutations per patient by Sanger sequencing. These APL specific mutations affected not only known mutational targets in leukemia, such as WT1 and KRAS, but also genes with potential implications in leukemogenesis, such as LYN, encoding a kinase which acts downstream of FLT3, and a novel homeobox gene. Our findings demonstrate that exome sequencing is an efficient method to screen for leukemia specific point mutations and small indels that may collaborate with the PML/RARA fusion during the onset and progression of APL.

0027

HIGHLY EXPRESSED MIR-125B AND LET-7C FORM ONE POTENTIAL MECHANISM TO EVOLVE THE GROWTH INHIBITION OF TGFB IN ACUTE MEGAKARYOBLASTIC LEUKEMIA OF DOWN SYNDROME PATIENTS

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Background. TGFB has been shown to play a crucial role in cancer development through its function as a tumor suppressor. Via the SMAD-signaling pathway it regulates apoptosis as well as differentiation. In hematopoiesis TGFB is mainly known through its inhibitory effects on proliferation. Children and neonates with Down syndrome (DS; i.e. trisomy 21) are highly predisposed to develop acute megakaryoblastic leukemia (ML-DS) and the antecedent transient leukemia (TL-DS). Significantly increased levels of TGFB in the amniotic fluid of DS-pregnancies and the secretion of TGFB through the leukemic megakaryoblasts are suggestive for a pivotal escape mechanism of the leukemic cells to evade the growth inhibition of TGFB. The molecular mechanism of this disruption of the TGFB pathway have still to be elucidated in ML-DS. Especially the role of the highly expressed chromosome 21 (hsa21)-encoded miRNAs miR125b-2, miR99a and let-7c in this TGFB escape mechanism of ML-DS cells remains unknown. Aims. Here, we studied the function of hsa21-encoded miRNAs miR-125b-2, miR-99a and let-7c in this TGFB escape mechanism. We hypothesized a putative role of these miRNAs in DS leukemogenesis by suppressing its inhibitory effects allowing ML-DS-blasts to grow under high TGFB conditions. Methods. We employed pathway-specific in silico target prediction (Diana mirPath and miRGen), followed by in vitro studies to assess the response of various cell lines to TGFB and by functional validation in lentivirally transduced cell lines. Results. Two independent target prediction algorithms showed in silico enrichment of TGFB pathway components in the potential targetome of the Hsa21 encoded miRNAs, especially miR-125b-2 and let-7c. We addressed our attention to identify the key factors of the disruption of the TGFB signaling, which seems to be negatively regulated through the miRNAs. Consistent with these data we found decreased mRNA levels for SMAD3 (~10fold in ML-DS cell lines compared to K562 cells exhibiting low endogenous hsa21 miRNA expression), for TGFBR1 (~5fold compared to K562) and for SMAD2 and SMAD4. Next, we assessed the viability of amongst others leukemic cell lines with known TGFB-escape mechanisms, ML-DS and non DS-AML cell lines with various levels of miRNA expression under a wide range of TGFB concentrations. These studies gave high indices that the TGFB resistance is caused by miR-125b-2 or let-7c. Thus, we focussed on two TGFB-responder cell lines, NB4 (FAB-classification M3) and MV4-11 (FAB-classification M5). Overexpression in NB4 and MV4-11 with decreased basic levels of the respective miRNAs conferred a stronger survival (up to 1.4fold increase of viable cells after 5 days of TGFB-incubation relative to controls) under high TGFB concentration. Summary. Our data revealed the connection between the phenotypic non-responder morphology of the ML-DS blasts and miRNA expression levels. We could demonstrate the interplay of miR-125b-2, let-7c and TGFB signaling showing the morphological change of high sensitive cell lines into more resistant cell lines through overexpression of miR-125b-2 in MV4-11 and through overexpression of miR-125b-2 and let-7c in NB4 cells.

0028

HIGH-THROUGHPUT CLONAL SEQUENCING OF SMALL RNA TRANSCRIPTOME IN ACUTE MYELOID LEUKEMA CHARACTERISED HUNDRED OF NOVEL SMALL RNA MOLECULES

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Background. Despite many years of clinical research into the treatment of acute myeloid leukaemia (AML), there is still a real need for new therapeutic targets. Because of their regulatory role in gene expression, microRNAs (miRNAs) and other small non-coding (snc)RNAs could potentially be exploited in clinical treatment regimes. However, a deeper understanding of their role in AML needs to enable this. It was recently established by real-time PCR that the expression level of miRNAs provides molecular signatures characteristic of the major translocation-mediated gene fusion events in AML. However the results were restricted to a fraction of known miRNAs. Aims. This study aims to systematically characterise the whole small RNA transcriptome in AML. This includes the quantification and the discovery of novel tu- mor associated miRNAs and sncRNA species, including edited mole- cules, relevant to the occurrence, development and management of the disease. Methods. We report the results of high-throughput clonal sequencing (Solexa-Illumina) of 38 libraries of size-fractionated RNA obtained from 36 cyto- genetically and clinically distinct cases of AML and 2 normal bone marrow from healthy donors. Sequences were aligned to the genome of reference (NCBI36/hg18) and to miRNA and sncRNA databases (MiRBase and RNAdb) using Novoalign. A location was recorded where >=2 reads mapped in any sample. The distribution of sncRNAs and miRNAs was determined for each sample. MiRDeep and snoSeeker were used to identify potential novel miRNA and small nu- cleolar RNA (snoRNA) candidates, respectively. Results. We detected the expression of 765 known miRNAs and 684 uniquely located species of snoRNAs, including small nuclear (sn)RNAs, piwiRNAs, Y-RNAs, and small nucleolar (sno)RNAs. MiRNAs and other snoRNAs accounted for 53.8% and 21% of total reads, respectively. Alignment of the sequence to the hairpin miRNA database confirmed the presence of 144 miRNA star (*) not yet reported in the miRBase database. Approximately 3.7% of total reads were from unknown tags and were inves- tigated for novel miRNA and snoRNA species. A total of 575 potential miRNA candidate hairpins were identified. Of these, 126 were located in exonic regions of the genome. We also identified 37 C/D Box and 51 H/ACA Box, potential novel snoRNAs. In general, the novel snoRNAs were expressed at a low level compared with the already known mole- cules. ANOVA test applied to the 38 AML samples in order to find miRNAs with statistically significant differences in expression level among the major cytogenetic groups identified 177 miRNAs passing a 5% significance level. Conclusions. High-throughput sequencing revealed a complex distribution of small non-coding RNAs, including the detection of new hairpin molecules, in cancer cells. Also it provided the small RNA expression pattern in AML. Further investigations, especially in the role of snoRNAs, may uncover novel aspects of the disease aetiology.

London, United Kingdom, June 9 – 12, 2011

haematologica | 2011; 96(s2) | 11
TET2 AND IDH1/2 MUTATIONS IN SECONDARY ACUTE MYELOID LEUKEMIAS: A FRENCH RETROSPECTIVE STUDY

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Background. TET2 mutations were recently involved in 20-26% of MDS, in 12% of MPN and in 10-17% of AML. The impact of TET2 mutations is not fully examined in secondary AML (SA) which encompass myelodysplasia-related changes (MRC) AML and therapy-related (TR) AML according to the WHO classification. In this study, we have analyzed marrow samples from 247 patients at diagnosis identified as SA. We have determined the status of TET2 gene together with other genes frequently mutated in AML (IDH1, IDH2, NPM1, FLT3, N and K-RAS, c-KIT). Results. The cohort of 247 samples was subdivided in 201 MRC AML and in 46 TR AML. A normal karyotype (NK) was found in 70 patients (39.5%). 69 abnormalities of the TET2 sequence were found and dispatched among 49 patients harboring a heterozygous TET2 mutation leading to a 19.8% of patients with at least one TET2 mutation. TET2 mutations are significantly more frequent in MRC AML (22.3%) than in TR AML (8.7%) (P=0.03). The SA patients harbouring a TET2 mutation are significantly older and present higher levels of hemoglobin and leukocyte, involving monocytes, than unmutated patients. They also exhibit a significant lower MCV and platelets count. Percentage of blasts in the bone marrow is similar in the two groups. These particular modifications of the biological parameters are observed independently of the presence of a normal karyotype. A NK is present in 51% of TET2 mutated patients and in 25% of unmutated patients, indicating that TET2 mutations are strongly associated with NK (P<0.001). By contrast, 57% of unmutated patients versus 29% of TET2 mutated patients have a complex karyotype. IDH mutations (~14% of the whole cohort) are not mutually exclusive of TET2 mutations. No statistical association can be found between TET2 mutations and NPM1, FLT3, N and K-RAS mutations. There is a slight increase of c-KIT mutations in the TET2 mutated group as described in mastocytosis. In 158 patients receiving intensive chemotherapy at diagnosis, the complete remission rate and the overall survival are identical in the TET2 mutated and unmutated groups. Conclusion. TET2 mutated SA at diagnosis present particular clinical and biological characteristics but no association with markers of poor prognosis as complex karyotype or FLT3 mutations. TET2 mutations have no impact on survival.

DELETION OF THE TUMOR SUPPRESSOR GENE NF1 OCCURS IN 5% OF MYELOID MALIGNANCIES AND IS ACCOMPANIED BY A MUTATION IN THE REMAINING ALLELE IN 56% OF CASES

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Background. Alterations of the RAS pathway play an important role in the pathogenesis of myeloid malignancies and occur either by activating mutations in RAS itself or by mutations in genes involved in RAS-dependent pathways such as FLT3, KIT or CBL. The NF1 gene encodes neurofibromin 1 and is a negative regulator of RAS signaling. Aims/Methods. In order to analyze the role of NF1 in adult AML, we first evaluated NF1 gene expression in 272 AML using microarrays (HG-U133 Plus 2.0, Affymetrix, Santa Clara, USA). These included cases with t(15;17) (n=15), t(8;21) (n=16), inv(16) (n=7), t(11q23)/MLL-rearrangement (n=10), inv(3)(t;3;5) (n=5), complex karyotype (n=47), normal monocytes, and with mutated or heterozygous abnormality (n=77). Results. The median NF1 expression intensity was 131.6. 68 cases showed an expression intensity of NF1 below 98.6 (first quartile, low expression group). In this cohort cases with t(8;21) (n=10) or complex karyotype (n=18) were over-represented (Chi-square: p<0.0001 and p=0.001, respectively), while cases with normal karyotype (n=16) were under-represented (Chi-square: p=0.016). Low NF1 expression could be due to a deletion of one NF1 gene copy. Therefore, we performed FISH analysis using a NF1-probe in 54/68 of cases with low NF1 expression with available material. In 11/54 of these cases (20.4%) a NF1 deletion was identified by FISH. Chromosome banding analysis in 54 cases with NF1 deletion revealed a complex karyotype (n=7), one of these cases with inv(3), a normal karyotype (n=2), an inv(3) (n=1), and a 5q-deletion accompanied by +21 (n=1), respectively. To further investigate the incidence of NF1 deletion in myeloid malignancies, 889 additional patients were analyzed by FISH for NF1 deletion. A heterogeneous NF1 deletion was observed in 46/889 (5.2%) patients. In detail, 23/315 (7.3%) of novo AML, 7/42 (16.7%) t-AML, 7/176 (4.0%) CML, 2/165 (1.2%) MDS, and 7/136 (5.1%) MPN showed NF1 deletions. Chromosome banding analysis in the NF1-deleted cases revealed a normal karyotype (6/509, 1.2%), an inv(16)(t;16;16) (6/57, 16.2%), an inv(3)(t;3;5) (3/27, 11.1%), a complex karyotype (n=19/62, 30.6%) or other abnormalities (12/62, 19.7%). The frequency of NF1 deletions was remarkably high in patients with inv(16)(t;16;16) and inv(3)(t;3;5) (6/37 cases (16.2%) and 3/27 cases (11.1%), respectively), both subgroups are known to be associated with N Ras mutations. The frequency of NF1 deletions in patients with complex karyotype was 30.6% (19/62), and was elevated in patients with normal karyotype 1.2% (6/509). Further, next-generation deep-sequencing (54 Life Sciences, Branford, CT) was used to investigate molecular NF1-mutations in 32 patients with NF1 deletions. In 18 cases (56.3%) a NF1 mutation was detected. Conclusions. NF1 deletions occur in 5% of de novo AML, 5% of MDS, and 5% of MPN and are therefore a more frequent and important genetic mechanism for activating the RAS pathway in adult myeloid malignancies. Furthermore, in 58% of cases with NF1 deletion as detected by FISH a NF1 mutation was observed in the remaining allele. Future studies are necessary to determine the prognostic impact of NF1 deletion either caused by oncogenic deletion, deletion and/or mutation in the various subtypes of myeloid malignancies.

NPM1 DELETION/HAPLOINSUFFICIENCY IS A FEATURE OF MYELOID LEUKEMIAS WITH HIGH RISK CYTOGENETICS

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Background. We previously identified heterozygous NPM1 exon 12 somatic mutations in approximately 60% of adult acute myeloid leukemia (AML) with normal karyotype (NEJM 2005). The unmutated allele was never deleted in AML mutated cases. NPM1 haplo-insufficiency leads to genomic instability and causes bone marrow dysplasia in mice (Nature 2005). NPM1 maps at human chromosome 5q35. Aims. To investigate NPM1 haploinsufficiency in human myeloid leukemias with complete or partial loss of chromosome 5 (5-5q). Methods. A total of 213 bone marrow or peripheral blood samples were studied from 120 females and 93 males (age range 9-94) with myeloid leukemias and -5/5q- or not. Findings from 145 cases have already been published (PLoS One 2010). The other 68 cases [39 myelodysplastic syndrome (MDS), 27 AML, and 2 primary myelofibrosis (PMF)] belong to a consecutive prospective series of cases that were analyzed in our laboratory from January 1° 2007 to January 31° 2011. Cytogenetically, 65/213 cases had isolated 5q-; 127 cases had a -5/5q- plus one or more changes; 21 cases had a complex karyotype without -5q-. To investigate NPM1/5q55 we used interphase FISH with clone RP11-117L6 encompassing the entire gene. TP53, ATM, and XRCC2 deletions were investigated by FISH with RP11-199F11 clone, LSI p53/LSI ATM (Vysis, Abbott), and CTD-2326K14. In 31 prospective cases with NPM1 haploinsufficiency we further investigated the incidence of the residual allele were alternated by direct sequencing. Results. NPM1 heterozygous deletion was found in 58 cases: 56/127 (44%) with non isolated -5q- and 2/65 (3%) with isolated 5q- (P=0.000). According to NPM1 status, cases with non-iso-
labeled -5/-5q were grouped as NPM1+/- and NPM1+-+. A diploid karyotype was present in 2/56 NPM1+- (3.5%) and in 23/71 NPM1+-+ (52.3%) (P=0.001). Significantly more monosomies were found in the NPM1+- subgroup (median: 3, range: 0-9 vs median: 2, range:0-7) (P=0.009). Gross chromosomal changes, i.e. markers, rings, and double minutes, were found in 43/56 NPM1+-+ (76.7%) and in 33/71 NPM1+-+ Base (46.4%) (P=0.0005). Distribution of events involving TP53, ATM, 5q25 or XRC37/2q36 was not significantly different in NPM1+-+ and NPM1+-+ subgroups. No cryptic deletion emerged in cases with complex karyotype without -5/-5q.

Summary/Conclusions. NPM1 deletion/haploinsufficiency clearly emerged as a feature of over 40% of human myeloid leukemias associated with high risk cytogenetics including -5/-5q-, with the incidence reaching 41.9% in the prospective series of 68 cases. NPM1+/- cases showed a significantly higher prevalence of monosomies and gross chromosomal changes, suggesting that in humans, as in mice, NPM1 haploinsufficiency was related to genomic instability independently of NPM1+/- deletion/haploinsufficiency and normal karyotype. As far as we know, NPM1+/+ (46.4%) vs NPM1+/- (46.4%) (P=0.0005). Distribution of events involving TP53, ATM, 5q25 or XRC37/2q36 was not significantly different in NPM1+-+ and NPM1+-+ subgroups. No cryptic deletion emerged in cases with complex karyotype without -5/-5q.


0032 REACTIVATING PP2A BY FTY720 AS A NOVEL THERAPY FOR AML WITH C-KIT TYROSINE KINASE DOMAIN MUTATION

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Background. C-KIT is a type 8 receptor tyrosine kinase. Its tyrosine kinase domain (TKD) mutations are associated with poor prognosis in acute myeloid leukemia (AML). The most frequently occurred C-KIT/TKD mutation in AML is D816V. Protein phosphatase 2A (PP2A) is a human tumor suppressor and its dysfunction might contribute to malignant cell behavior. As far as we know, PP2A activity status in malignant cells has not been identified. FTY720 shows promising preclinical activity in patients with refractory CML, while its role in C-KIT/TKD AML remains to be elucidated. Aims. To investigate PP2A activity in C-KIT/TKD AML and develop FTY720 as a novel therapy for C-KIT/TKD AML. Methods. Eight AML patients harboring C-KIT/DT16V and twelve AML patients harboring wide-type C-KIT (C-KIT/WT) were recruited and informed consent was obtained. Real-time quantitative PCR was used to identify C-KIT/WT and C-KIT/DT16V. PP2A activity in the peripheral blood mononuclear cell of patients was assayed with a PP2A Immunoprecipitation Phosphatase Assay Kit. Furthermore, primary AML cells and cell lines were treated with FTY720 alone or combined with specific PP2A inhibitor okadaic acid (OA) in culture. Cell growth was assessed using the CCK8 Kit. Cell apoptosis was analyzed by flow cytometry after dual staining with annexin V propidium iodide. Western blotting was used to analyze the protein changes. All experiments were repeated three times. Statistical analysis was performed using SPSS 17.0. Results. PP2A activity was significantly decreased in AML subgroups harboring C-KIT/DT16V mutation compared with C-KIT/WT AML (P = 0.045). In addition, FTY720 induced toxicity in all AML cells, including Kasumi- I, HL60 harboring C-KIT/WT and primary cells from AML patients in a time- and dose-dependent manner. PP2A inhibitor OA rescued these cells from FTY720-induced apoptosis. Furthermore, increased PP2A activity was detected after FTY720 treatment. When cells were pretreated with OA, PP2A activity was partially rescued. Finally, PP2A expression remained unchanged after FTY720 treatment. Our results strongly suggested that FTY720-induced cytotoxicity was mediated by PP2A activation without altering its expression. Interestingly, it was observed that cells harboring C-KIT/TKD mutation were more sensitive to this agent (IC50: 15.5 µM VS 20 µM, P<0.05). Conclusions. From the standpoint of prevalence, PP2A activity is decreased in patients with C-KIT/TKD AML. The study also provides evidence for the PP2A-dependent toxicity of FTY720 in AML cells, and these effects of FTY720 appear to be most marked in C-KIT/TKD AML where conventional chemotherapeutic approaches are most likely to fail. The significant preclinical efficacy of FTY720 indicates that it may be valuable therapeutic agents for refractory C-KIT/TKD AML.
0034 THERAPY-INDUCED SELECTIVE ELIMINATION OF LEUKEMIA INITIATING CELLS IN XENOTRANSPLANTATION MODEL OF AML

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Background. Acute Myeloid Leukemia (AML) is a heterogeneous disease associated with various genetic and epigenetic abnormalities. It is characterized by the uncontrolled proliferation of myeloid blast cells blocked in their differentiation. Epigenetic abnormalities are reversible in contrast to genetic abnormalities, which are irreversible. Agreeing on these concepts it was argued that epigenetic targeting possibly would bring back the malignancy to a more normal state. 5-azacytidine (5AZA, DNA methyltransferases inhibitor) and valproic acid (VPA, Histone deacetylases inhibitor) are among the various pharmacological drugs used as epigenetic modifiers in clinics to treat AML.

Based on these reports, we hypothesized that combining these drugs along with a differentiating and apoptotic agent ATRA may have good therapeutic effects in all AML subtypes including ATRA resistant AML.

Aims. Establishment of a preclinical model for the validation of drugs in targeted therapy of AML. Methods. Blood and bone marrow samples from patients were collected after informed consent was obtained. CD3 negative cells were transplanted through retro-orbital sinus into leukemic human cells, as the remaining human cells after the treatment were mature and differentiated. In case of Acute promyelocytic leukemia (APL) there was decrease in the PML-RAR transcript after treatment, clearly indicated the decrease in the leukemic blast in the mice. Interestingly, with the decrease in human cells in the treated mice, there was a restoration of the mouse hemopoiesis suggesting that there is no toxic effect of the drug combination on normal hemopoiesis and the hematopoietic microenvironment.

Conclusion. This is a useful tool to validate the real effectiveness and the presence of leukemic initiating cells after treatment. These results also show that as in a human setting an effective therapeutic effect of this drug combination may be obtained in xenotransplanted mice. The xenotransplantation model provides an appropriate system to study and optimize drugs before entering to clinics. Email: patelsatyana@gmail.com

0035 DNMT3A MUTATIONS IN INTERMEDIATE-RISK ACUTE MYELOID LEUKEMIA

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Background and Aims. Recently, mutations in DNMT3A gene were found to cause worse survival of patients with AML. We attempted to test their impact in our patient cohort. Patients and methods. 165 AML patients with an intermediate-risk cytogenetic profile, diagnosed between years 1999 and 2008, were screened for the presence of mutations in DNMT3A gene. The male/female ratio was 74/89. The median age at diagnosis was 56.1 (18.2-81.7) years and the initial median WBC count was 23.5 x 109/L (0.7-483.7). RT-PCR followed by direct sequencing (exons 11-26) was used to test the presence of DNMT3A mutations. Results. 44 patients out of 165 (27.0%) carried a mutation in DNMT3A gene. 28 of them had a single nucleotide change, compared with Arg882 (21 had Arg882His, 6 Arg882Cys and 1 had Arg882Ser). 11 patients had another missense mutation while the remaining 5 carried various frameshift mutations. The incidence of mutations was slightly higher among female patients (30.3% vs. 23.0%) and was independent of the age at presentation. According to the FAB classification, DNMT3A mutations were most frequently present at subtypes M1 (14/57; 37.8%) and M4 (11/35; 31.4%), on the other hand, none of 9 patients with M5 subtype harbored the mutation. Occurrence of DNMT3A mutations was associated with the presence of FLT3/ITD (P=0.012). Patients with DNMT3A mutation had significantly higher initial WBC compared to those without mutation (47.5 x 14.6 x 109/L; P=0.0004). There was no difference between mutated and unmutated group in reaching complete remission (CR) (52.3% vs. 54.6%; P=0.395). Patients with any change of codon Arg882 more easily reached CR than those with any other DNMT3A mutation (57.1% vs. 43.8%; P=0.196). The CR rate within the DNMT3A-mutated group was unfavorably affected by the presence of FLT3/ITD (62.5% vs. 40.0%; P=0.068). Patients positive for DNMT3A mutation significantly more often suffered a relapse (60.0% vs. 40.8%; P=0.049). Among mutated patients, cases with Arg882 aberration relapsed less often than patients with any other mutation (53.3% vs. 57.1%; P=0.124). The overall survival (OS) was not affected by DNMT3A mutations (10.9 months in mutated vs. 13.6 in unmutated cases; P=0.886). Within the group of FLT3/ITD positive cases, DNMT3A mutations caused shorter OS (6.09 vs. 10.24 months; P=0.012). When DNMT3A-positive cases were analyzed with respect to the FLT3/ITD status, those carrying both of these aberrations had significantly shorter OS than FLT3/ITD negative ones (6.09 vs. 18.87 months; P=0.002). Out of 20 patients harboring both of these mutations only one is still alive. Hematopoietic stem cell transplantation (HSCT) could salvage DNMT3A-mutated patients. 5/11 patients survived after allo HSCT. Conclusion. We have confirmed high incidence of DNMT3A mutations in patients with AML with an intermediate cytogenetic risk. Patients with any mutation in DNMT3A gene tend to relapse more often than negative cases. Double-mutated (FLT3/ITD+DNMT3A) patients have a very poor prognosis. Patients with AML harboring DNMT3A mutation may benefit from HSCT.

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targets known to confer G2/M-arrest (p21, p27) as well as apoptosis (PUMA). To delineate the functional consequences of these observations, we recapitulated experiments in myeloid cell lines in the presence of biochemical cell cycle inhibitors. We thus show that both agents increase activation of the checkpoint-kinases-1 and -2 (phosphorylation of Chk-1Ser17 and Chk-2Ser68) as well as their downstream targets, the Chk1 mediated by UCN-01 (and a lesser degree inhibition of Chk-2 by NCI-108558) abrogated G2/M-arrest induced by hypomethylating agents and showed that hypomethylating agents can correct aberrant signaling pathways in MDS/AML, notably by correcting aberrant cytoplasmic localization of FOXP3a thereby (re)establishing its function as a transcriptional regulator of cell-cycle-arresting and apoptosis-inducing molecules.

0037
MIR-155 UP-REGULATION IN FLT3 POSITIVE AML INVERSELY CORRELATES WITH EXPRESSION OF MYELOID-SPECIFIC TRANSCRIPTION FACTORS PU.1 AND CEBPβ
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Background. Acute myeloid leukaemia (AML) arises from myeloid progenitor cells that are arrested at early stages of differentiation. It is a cytogenetically heterogeneous disorder with acquired recurrent chromosomal alterations detected in about 55% of adult patients, such as translocations, inversions, deletions, trisomies, and monosomies. In the remaining 45% of cases of normal karyotype AML, a number of novel molecular abnormalities have been described, such as the internal tandem duplication (ITD) or mutation (D835) of FLT3 gene, mutations of NPM1 gene, mutation of CEBPA gene and partial tandem duplication of the MLL gene. Several studies have shown that genome-wide gene expression profiling can clearly distinguish the major cytogenetic groups, so providing a better understanding of the underlying disease biology. Despite this progress, focusing on known genes will likely not suffice to uncover the molecular puzzle of AML. The integration of a whole genome approach including non-coding RNAs may lead to an improved understanding of AML biology. Despite this progress, focusing on known genes will likely not suffice to uncover the molecular puzzle of AML. The integration of a whole genome approach including non-coding RNAs may lead to an improved understanding of AML biology. Despite this progress, focusing on known genes will likely not suffice to uncover the molecular puzzle of AML. The integration of a whole genome approach including non-coding RNAs may lead to an improved understanding of AML biology.

Methods. We performed quantitative real-time RT-PCR to study the expression of specific mRNAs by either translational inhibition or mRNA degradation. There are several indications that miRNAs might be a new class of genes involved in human cancer and has been observed that distinct patterns of miRNA expression reflect different developmental stages and different genetic categories of AML. Our previous work has demonstrated that miR-155 directly represses a broad range of target mRNAs implicated in myeloid hyperplasia and/or hematopoiesis.Using available prediction target algorithms we selected hypothetical miR-155 regulated genes such as PU.1 and CEBPβ, both genes codify for lineage specific transcription factors, indispensable for normal myeloid development. In the same cohort of 97 patients we found an inverse correlation between mir-155 expression levels and its predicted targets: PU.1 (Fold=0.471; P=0.005), CEBPβ (Fold=0.414; P=0.008). Conclusions. Based upon our current study, miR-155 appears to play a role in malignant haematopoiesis targeting the expression of central myeloid specific transcription factors that may contribute to block differentiation. Future functional analysis will clarify better the role of mir-155 in AML genesis and its molecular mechanism in the inhibition of myeloid differentiation.
characterized, this has not translated into new therapeutic strategies with notable improvements in overall disease-free survival. Heat shock proteins (HSPs) exhibit sophisticated cellular protection mechanisms, acting as molecular chaperones that prevent the formation of protein aggregates and assist proteins in their folding to native structures. HSPs are important for the regulation and remodeling of several proteins involved in leukemogenesis (e.g., several transcription factors and kinases), and expression of HSP90 has been considered as a promising therapeutic strategy. Aims. We hypothesized that the HSP90 level varies between AML patients, and that this variation can be used for subclassification of patients with regard to responsiveness to targeted therapy. The aim of the present study was to quantify the HSP levels for a large cohort of AML patients, and to compare HSP levels and the expression of the HSP90 inhibitor 17-dimethylaminoethylamino-17-demethoxygeladanycin (17-DMAG) in these patients. Methods. We included 75 consecutive patients. AML cells were isolated by density gradient separation and contained at least 95% leukemia blasts. Cell lysates were prepared from cryopreserved cells and protein levels of HSP27 (phospho-Ser15, phospho-Ser82), HSP40, HSP60, HSP70 and HSP90 determined by multiplex immunos assay for flow cytometric analyses. The levels were compared with clinical and biological patient characteristics by using robust bioinformatic tools including hierarchical clustering analyses. Functional characterization of 17-DMAG effects on AML cells included investigation of apoptosis, proliferation and expression of angioregulatory cytokines. Results. AML cells derived from various patients differed in the expression level of different HSPs, and patients could be divided into two major clusters with low (cluster I) and high (cluster II) HSP levels. The two clusters did not differ with regard to AML etiology (de novo vs. secondary/relapse), differentiation (FAB classification, expression of CD34), cytogenetics or frequency of NPM1 mutations. In contrast, the frequency of FLT3 mutation was significantly higher in the high-HSP cluster II, thus the HSP levels are generally higher in primary AML cells with chemoresistant FLT3 mutations. These findings provide insights into the pathogenesis of RUNX1 mutated AML M0 (Silva et al., Blood, 2009) indicating that the expression of these genes is very likely to be influenced by RUNX1 mutations. These findings provide insights into the pathogenesis of RUNX1 mutation positive AML and may contribute to the identification of novel diagnostic markers and targets for therapy.

Figure 1. DNTT-Expression in RUNX1 wt vs RUNX1 mutated AML.

0040
THE DEACETYLASE INHIBITORS DACINOSTAT AND VORINOSTAT INHIBIT SELF-RENEWAL AND REPOPULATION CAPACITY OF AML1/ETO- AND PLZF/RARA-EXPRESSING MURINE STEM CELLS

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Background. Deacetylase inhibitors (DACi) are promising drugs leading to growth inhibition, cell cycle arrest, premature senescence and apoptosis in malignant cells. In acute myeloid leukemia, the most primitive population of leukemic stem and progenitor cells (LSC) are thought to account for relapses of the disease. As the efficacy of a DACi therapy depends on its capacity to eradicate the LSC compartment, we aimed to study function and gene expression in response to DACi treatment. Aims. Aim of our study was 1) to analyze the impact of an in vitro treatment with various DACi on the proliferation and self-renewing capacity of normal and leukemic stem cells and 2) to assess DACi-induced regulation of genes involved in survival of leukemic progenitor cells. Methods. Murine Sca1+/lin- hematopoietic stem cells (HSC) were retrovirally infected with AML1/ETO and PLZF/RARα known to induce an acute leukemia in C57BL/6 mice. Serial replating and day 12 spleen colony-forming unit assays (CFU-S) were performed to assess the impact of DACi on the functional capacity of LSC. For gene expression studies PLZF/RARα, AML1/ETO- and mock-transduced 2D cells were analysed by Westernblot after treatment with valproic acid (VPA, 150µg/ml), dacinostat (2.5-20mM) or vorinostat (1 or 2µM). Results. Sca1+-lin- HSC carrying AML1/ETO or PLZF/RARα had a serial replating capacity far exceeding that of mock infected controls (at least 6 vs. 2 rounds of plating, resp.). Of note, continuous presence of DACi in the methylcellulose resulted in a progressive loss of colony forming cells suggesting exhaustion of leukemic progenitors over time. The replication capacity of mock- as well as AML1/ETO- or PLZF/RARα-transduced HSC was analysed after a 7-day culture period in presence of cytokines +/- DACi. Compared to control cultures, the class I DACI VPA induced an up to 3-fold expansion whereas the more potent class II DACI dacinostat and vorinostat lead to a considerable cell loss by at least one log. When all the progeny grown were injected into lethally irradiated mice, the sparse dacinostat- and vorinostat-treated cells gave rise to a significantly lower number of spleen colonies compared to only cytokine- or VPA-treated controls (n=5 independently). In the spleen of mice transplanted with AML1-ETO and PLZF/RARα-transduced HSC, oncocogene expression was detected by qPCR. Dacinostat seemed to exert a deleterious effect on LIC, as we failed to detect an AML1/ETO- or PLZF/RARα-signal. VPA turned out to be effective in PLZF/RARα-, but not AML1/ETO-positive LIC and vorinostat gave conflicting results. In mock transfected Sca1+-lin- HSC, dacinostat and vorinostat seemed to damage normal committed progenitor cells as measured by serial replating, but largely

The AML1/RUNX1 gene is a frequent mutational target in myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). We have screened 95 cytogenetically normal AML (CN-AML) patients for RUNX1 mutations by capillary sequencing of genomic DNA. This led to the identification of 9 patients with RUNX1 mutations (9.5%). While RUNX1 mutations were significantly associated with older age, male sex, MLL-PTD mutations and poor clinical outcome, an inverse correlation with NPM1 mutations was observed. Patients with RUNX1 mutations showed a unique gene expression pattern with differential expression of 55 genes. The most significantly upregulated gene in RUNX1 mutated CN-AML was DNTT (deoxynucleotidyltransferase, terminal) which was previously shown to be associated with poor prognosis in AML (Venditti et al., Leukemia, 1998). DNTT plays a role in early lymphoid differentiation where it generates antigen receptor diversity by synthesizing non-germ line elements (N-regions) at the junctions of rearranged Ig heavy chain and antigen receptor gene segments. Thus, high expression of DNTT in RUNX1 mutated AML might indicate abortive lymphoid differentiation during leukemogenesis in these patients. Interestingly, 21 out of 55 differentially expressed genes were reported to be also deregulated in RUNX1 mutated AML.
spare the repopulating stem cell fraction responsible for establishing CFU-S. VPA significantly enhanced spleen colony formation as previously reported. Western blot of PLZF-RARAα AML1-ETO- and Mock-infected 32D cells showed a concentration-dependent downregulation of Bmi-1 and c-myc after treatment with VPA, dacinostat and vorinostat which may have contributed to vanishing of leukemic progenitors. Conclusion. In summary, our data suggest that in contrast to the class I DAcI VPA the potent class I DAcI dacinostat and vorinostat inhibit both proliferation and self-renewal of LIC.

0042 THERAPEUTIC POTENTIAL OF TARGETING KEY HOXA-TALE GENES IN AML
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Background. HOX genes are master regulators of hematopoietic stem and progenitor cell (HSPC) development. HOX interaction with TALE (PBX or MEIS) proteins is pivotal to their function. The prevailing hypothesis is that HOX/TALE expression underlies HSPC self-renewal while dysregulated HOX/TALE expression underpins maintenance of the leukemia initiating cell. Gene expression profiling studies support this hypothesis and highlight the importance of the HOXA cluster and TALE genes in hematopoiesis and leukemogenesis. HOXA6 and HOXA9 form part of the leukemia-associated ‘HOX code’ identified from human samples and murine models of this disease. Aims. To identify and therapeutically target the AML-associated HOXA-TALE axis in intermediate-2 (IM2) human AML. Methods. HOXA cluster and leukemia-associated TALE gene expression was evaluated in 145 favourable and intermediate AML patient samples by Affymetrix microarray and expression of the significantly different genes were confirmed by Q-PCR assays in an independent patient cohort. Immuno-precipitation assays were used to identify HOXA-TALE protein interactions using tags in overexpressing fibroblasts and endogenous interactions were determined AML cells. HOXA/TALE was transiently silenced using Nucleofection or viruses to package and deliver shRNA to OCI AML3 and U937 cells and knock-down their TALE partners were selected for further study. Specific shRNAs was identified as the most consistently and highly expressed ‘HOX code’ gene and their critical co-factors or modulation of downstream pathways could offer a novel strategy in the treatment of AML.

0043 THE ROLE OF CYSTEINE PROTEASES IN PAEDIATRIC LEUKAEMIA
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Background. Calpains and cathepsins are cysteine proteases known to be expressed in ALL and AML. We have previously shown that treatment with Calpain Inhibitor III (MDL28170) reduces cell viability and clonogenicity in a dose-dependent manner, demonstrating a more potent effect in cells carrying the AML1/MTG8 fusion gene (Kasumi-1) than in another M2 cell line (HL-60) lacking this translocation. MDL28170 also inhibits Cathepsin B and Cathepsin L. These lysosomal enzymes are both involved in the regulation of apoptosis, cell cycle progression and cell cycle control. They are highly expressed in many malignant cell types and their secretion has been identified as being an important mediator of solid tumour invasion and metastasis. The intracellular roles of these cathepsins and their effects in leukaemia are less well understood. Aims. We aimed to identify which calpains and cathepsins are important in leukemic maintenance and to functionally analyse their roles. We aim to test and validate these proteases as potential drug targets. Methods. Specific inhibitors of cathepsin B (CA074Me), Cathepsin L (Cathepsin L inhibitor 3) and Calpain 18/2 (PD150606) were chosen in addition to MDL28170 which inhibits all of the above proteases. Knockdown of cysteine protease expression was achieved using siRNA and confirmed using RTPCR and Western Blot. Cell-cycle analysis and apoptosis assays were also performed in the presence of the inhibitors. LD50 was determined using the WST-1 cell viability assay in t(8;21)(q22;q22) positive and negative cell lines, including some bearing the t(4;11) poor prognostic marker. The effects of Cathepsin L knockdown using shRNA were assessed. Results. Calpain and cathepsin inhibition has a dose-dependent cytotoxic effect in the AML and ALL cell lines tested. The effects of simultaneous calpain, cathepsin B and cathepsin L inhibition using MDL28170 are more pronounced than those seen when inhibitors meant to target specific cysteine proteases are used. Cathepsin inhibitors have a greater cytotoxic effect than calpain inhibitors. MDL28170, CA074Me and Cathepsin L Inhibitor 3 have a greater effect on Kasumi-1 clonogenicity than calpain inhibition in isolation using PD150606. Knockdown of the calpain small subunit and Calpain-2 using siRNA also reduced clonogenicity of Kasumi-1 cells by up to 80%. Summary/Conclusions. In summary, we show that calpains are important mediators of leukemic maintenance and clonogenicity. In addition, Cathepsins B and L are play a role in proliferation, clonogenicity and survival of AML1/MTG8 expressing cell lines and other leukemic populations. Further functional analysis is ongoing to examine the potential links between these pathways.

0044 ROLE OF DEATH CELL RECEPTORS PATHWAY IN APOPTOSIS INDUCED BY THE HISTONE DEACETYLASE INHIBITOR SODIUM BUTYRATE IN NPM1-MUTATED AML CELLS
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AML carrying NPM1 mutations [Falini B et al., NEJM 2005;352:254-266] accounts for about one-third of adult AML, shows distinctive biological and clinical features [Falini B et al., Blood 2007;109:874-885] and has been included as a provisional entity in the 2008 World Health Organization (WHO) classification of myeloid neoplasms. In spite of the relatively good prognosis of NPM1-mutated AML, there are still cases that show poorer outcome, especially those associated with FLT3-ITD mutation and elderly patient population. Therefore new therapeutic strategies needed to be explored. Here we investigated the effect of sodium butyrate, a short-chain fatty acid which has long been known to be a histone deacetylase inhibitor (HDACi) able to induce maturational normal and tumor cells, in cellular models of NPM1-mutated

Figure 1. Reduced HoxA6/A9 + chemotherapy reduces cell growth.
AML: i) the OCI/AML3 cell line, previously identified as a human AML cell line carrying cytoplasmic mutated NPM1 in the absence of FLT3-ITD; ii) primary AML cells originated from a patient with NPM1-mutated AML bearing FLT3-ITD mutation (M0NT1) and propagated as cell line in NOD/SCID mice; and iii) primary AML cells from 4 NPM1-mutated AML patients at diagnosis. In either cell lines or patients’ primary AML cells carrying NPM1 mutation, but not in the U937 or OCI/AML2 cell lines (not harboring NPM1 gene mutation) used as control, growth arrest and pro-apoptotic effects were evident after 24 hrs and marked after 48 hrs of treatment with doses of drug of 0.5-1 mM (Figure 1a and b). In particular, no signs of differentiation were evident at morphological examination of treated cells. Interestingly, induction of apoptosis was associated with activation of caspase-8 (Figure 1c), suggesting involvement of the death cell receptors pathway. Indeed, flow cytometric analysis showed increased expression of TRAIL-receptor DR5 upon drug treatment. Moreover, concomitant treatment with a specific caspase-8 inhibitor prevented cell growth arrest and markedly reduced apoptosis. Levels of either NPM1 mutant or wild-type protein did not appear significantly affected by treatment with ELP10’s anti-leukemic efficacy in AML-/MDS-derived cell lines was determined by retesting the drug impact on 32 selected cell lines (solid tumors, and leukemia cell lines) by employing a sulfonothio- damine B (SRB) assay. The exact dose-and time-dependency of ELP10’s anti-leukemic efficacy in AML/MDS-derived cell lines was determined by assessment of apoptosis (by staining with DIOC25(3) and FACS quantification) and cell cycle progression (PI staining and FACS quantification at 24h and 48h) on MDS/AML-derived cell lines (KG-1, KG-1a, HL-60, MOLM-13, Kasumi-1). Finally, ELP10’s anti-leukemic efficacy in vivo was characterized in Balb/c mice injected intraperitoneally with L1210 or P388 murine leukemia cells and treated with increasing dosages of ELP10 (ranging from 1.6mg/kg to 50mg/kg). Results. In the NCI cell line bank ELP10 exhibited anti-neoplastic activity in a large series of cell lines representative of solid tumors as well as leukemias. The anti-neoplastic activity was highest in cell line models of acute leukemia, notably of AML-derived cell lines, which exhibited the lowest IC50. Confirmatory experiments in a broad spectrum of MDS- and AML-derived cell lines established ELP10’s ability to induce time- and dose-dependent induction of apoptosis and cell cycle arrest in G2/M. Apoptosis- and/or cell cycle-arresting effects of ELP10 were observed - albeit to a different degree - in all tested myeloid cell lines, thus 72h of incubation of HL-60- or Kasumi-1 cells with 1µM ELP10 induced apoptosis in about 50% of cells, and increased G2/M-arrest by about 40% (24h) of cells. Finally, in Balb/c mice, ELP10 increased life-span by about 50% in P388-grafted mice, and by about 30% (low dose ELP10) to 90% (high dose ELP10) in L1210-grafted mice. Conclusions. These studies provide evidence for an in vitro and in vivo anti-leukemic activity of ELP10, especially in AML- and MDS-derived cells, even at low dose levels.

**0046**

**FUNCTIONAL CHARACTERIZATION OF THE PROMOTER REGION OF EVI1 IN ACUTE MYELOID LEUKEMIA**

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The EVI1 gene (3q26) codes for a transcription factor with important roles in development and leukemogenesis. Aberrant expression of EVI1 has been reported in patients with 3q26 rearrangements; however, about 9-26% of acute myeloid leukemia cases with no 3q abnormalities overexpress EVI1, and this is also associated with an unfavorable outcome. These rearrangements were the only known mechanism that led to EVI1 overexpression; although recently it has been described that MLL fusion proteins selectively activate EVI1 in HSC-derived MLL leukemic cells. Our aim was to functionally characterize the promotor region of the human EVI1 gene, and to identify transcription factors involved in its regulation. Material and Methods. Bioinformatic analysis, validation by chromatin immunoprecipitation (ChIP), siRNA analysis, and luciferase assays. Results. To determine the minimal promoter region, we generated seven 5’- truncation constructs of the EVI1 promoter upstream of the luciferase reporter gene. These constructs were transfected into HEK293T, HEL, HELPG2 and A549 cell lines. These experiments revealed that a 318bp region retains more than the 50% of the full length construct activity. To identify the upstream regulatory factors in this EVI1 minimal promoter region, we examined the 318bp sequence using MATCH, TFESEARCH and MatInspector softwares. Site-directed mutagenesis allowed us to define the contribution of the hypothetical binding sites found. Transfection of the mutated constructs in the HEK293T and A549 cell lines showed a marked decrease in the promoter activity when the ELK1 and RUNX1 binding sites were mutated. ChIP in HEL and HEK293T cell lines demonstrated that ELK1 and RUNX1 bind to the proximal promoter region of EVI1. Moreover, knockdown of RUNX1 and ELK1 by siRNA caused decrease of EVI1, demonstrating their involvement in the transcriptional regulation of EVI1. Taking together, this approach allowed us to identify a functional region of 318bp in the proximal promoter region of EVI1, with several binding sites for transcription factors important roles in hematopoesis. These results also showed that RUNX1 and ELK1 regulate the transcription of EVI1. Further studies to confirm the role of the transcription factors RUNX1 and ELK1 in acute myeloid leukemia cases with overexpression of EVI1 are in progress. E-mail address: mmaicas@alumni.unav.es

**Background.** ELP10 is a 9-hydroxyellipticine derivative namely 2(piperdino ethyl)-9-hydroxyellipticumine bicloride. Since it was developed as an anti-cancer agent, we here assessed its anti-neoplastic efficacy with particular emphasis on its anti-leukemic activity in MDS and AML. Methods. To delineate the anti-neoplastic activity of ELP10, a screening of 68 NCI-listed malignant cell lines was carried out. The IC50 was confirmed by retesting the drug impact on 32 selected cell lines (solid tumors, and leukemia cell lines) by employing a sulfonothiodamine B (SRB) assay. The exact dose- and time-dependency of ELP10’s anti-leukemic efficacy in AML/MDS-derived cell lines was determined by assessment of apoptosis (by staining with DIOC25(3) and FACS quantification at 24h, 48h and 72h) and cell cycle progression (PI staining and FACS quantification at 24h and 48h) on MDS/AML-derived cell lines (KG-1, KG-1a, HL-60, MOLM-13, Kasumi-1). Finally, ELP10’s anti-leukemic efficacy in vivo was characterized in Balb/c mice injected intraperitoneally with L1210 or P388 murine leukemia cells and treated with increasing dosages of ELP10 (ranging from 1.6mg/kg to 50mg/kg). Results. In the NCI cell line bank ELP10 exhibited anti-neoplastic activity in a large series of cell lines representative of solid tumors as well as leukemias. The anti-neoplastic activity was highest in cell line models of acute leukemia, notably of AML-derived cell lines, which exhibited the lowest IC50. Confirmatory experiments in a
Acute myeloid leukemia - Clinical 1

0047

STRATIFICATION ACCORDING TO TREATMENT RESPONSE IMPROVED OUTCOME IN CHILDHOOD ACUTE MYELOID LEUKEMIA

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Background. Treatment response is generally considered as prognostic factor; however, studies of minimal residual disease did not confirm independent significance. Since 2006 the AML-BFM study group stratified poor responders after 1st and 2nd induction to intensified therapy, either an additional element or eligibility for allogeneic matched donor stem cell transplantation (SCT). Methods. Seven hundred-ten children from the German AML-BFM trials 98 (n=346) and 2004 (n=364) were included. Excluded were children with acute promyelocytic leukemia (n=59), myeloid leukemia of Down syndrome (n=122), early death (n=53) or incomplete data (n=89), 280 children (52%) were treated in the SR-group* and 480 in the HR-group (68%). Complete remission (CR) was determined after induction, late complete remission (LCR) at the end of intensive therapy. Bone marrow smears were centrally analyzed during treatment. Immunophenotyping was performed to confirm the morphology results. In a 4-color approach, the following antibodies were used: CD33, CD34, CD2, CD7, CD56, CD15, CD117, CD123, CD13, and CD19. A correlation was found between morphology and immunophenotyping (Pearson R = 0.86). In 28% of the patients regenerating blasts could be distinguished from malignant blasts. Persistence of blasts was detected in 144 patients (20%); 53 patients showed more than 5% blasts (SR n=13, 2%, HR n=40, 5%) and 90 children more than 10% of blasts (SR n=13, 2%, HR n=77, 11%). Whereas in Study 98 only 13% of the SR patients were shifted to HR therapy, since 2006 it increased to 21%. In the HR patients with persistent blasts, SCT in 1st CR or PR increased from 22 to 53%. Vice versa, in patients without detectable blasts after 1st induction the frequency of SCT decreased from 9% to 6%. Overall, blasts persistence after 1st induction was associated with an improved event-free and overall-survival (EFS 35±5% vs. 53±3% plog rank 8x10-7; OS 56±4% vs. 76±2% plog rank 8x10-7). This was mainly an effect by the HR-group (EFS 35±5% vs. 53±3% plog rank 0.0004; SR: EFS 61±10% vs. 71±3% plog rank 0.12). Since 2006, the prognostic significance of “blasts after 1st induction” was lost (table 1).

Table 1.

Analyzing the effect of further therapy including SCT in patients with blasts after 1st induction, in study 98 36 out of 55 patients (65%) achieved CR and 41 (75%) LCR. This significantly improved in study 2004 (CR 71%, LCR 83% and since 2006 (CR 78%, LCR 95%, p<0.007). In conclusion, using treatment response for stratification, EFS and OS could be improved in childhood AML.

Reference


* Standard risk (SR) group: FAB M1/M2 with Auer rods/ t(8;21), FAB M4eo/inv(16) and blasts <5%; High-risk (HR) group definition: all others.
**0049**

**ADDITION OF PURINE ANALOGUE EITHER CLADRIBINE OR FLUDARABINE TO INDUCTION REGIMEN IS ASSOCIATED WITH IMPROVED SURVIVAL OF AML PATIENTS WITH HIGH-RISK KARYOTYPE**

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**Improvement of survival of AML patients with high-risk karyotype.** Fludarabine to induction regimen is associated with addition of purine analogue either cladribine or fludarabine to standard induction regimen based on daunorubicin and cytarabine. Conclusions: Prognosis of AML patients with unfavorable cytogenetic features is poor with reported long term survival of approximately 20% despite the use of alloHSCT. So far, any modifications of induction chemotherapy did not result in improved survival. Between 2004-2008 the Polish Adult Leukemia Group conducted a randomized trial comparing standard induction (daunorubicin 60 mg/m2 for 3 days + AraC 200 mg/m2 for 7 days) vs. the same regimen combined with either cladribine 5 mg/m2 on days 1-5 (DAC) or fludarabine 25 mg/m2 on days 1-5 (DAF). Inform consent was obtained. The goal of the current analysis was to evaluate if the addition of purine analogue affects outcome in a cohort of patients with high-risk karyotype, as defined according to SWAG criteria. Among 652 patients with newly diagnosed AML included in the PALG DAC vs. DAF vs. DA study, 111 presented with unfavorable karyotype. The median age was 50 (19-60) years. 35 patients were randomly assigned to DA arm, 35 to DAC, and 41 do DAF. In the respective study groups the rate of CR was 37%, 60%, and 54% (DA vs. DAC+DAF; p=0.05). Among CR patients 41% received consolidation followed by either HLA-matched related or unrelated donor (DA, 23%, DAC 48%, DAF 45%). With the median follow-up of 2.8 years the 3-years probability of the overall survival was 20% for DA arm, 56% for DAC and 57% for DAF (DA vs. DAC+DAF; p=0.01) (see: figure). Leukemia-free survival equaled 45%, 31% and 56%, respectively (p=NS). No significant differences could be demonstrated with regard to remission duration and non-relapse mortality. All three regimens were characterized by comparable hematological and non-hematological toxicity. We conclude that the addition of purine analogue either cladribine or fludarabine to standard induction regimen based on daunorubicin combined with AraC is associated with improved survival of patients with newly diagnosed AML and high-risk karyotype. The advantage is mainly a consequence of increased rate of complete remission.

**0050**

**MANAGEMENT AND OUTCOME OF OLDER PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) IN FIRST RELAPSE: A STUDY FROM THE ACUTE LEUKEMIA FRENCH ASSOCIATION (ALFA)**

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**Aim.** Prognosis of AML patients with unfavorable cytogenetic features is poor with reported long term survival of approximately 20% despite the use of alloHSCT. So far, any modifications of induction chemotherapy did not result in improved survival. Between 2004-2008 the Polish Adult Leukemia Group conducted a randomized trial comparing standard induction (daunorubicin 60 mg/m² for 3 days + AraC 200 mg/m² for 7 days, DA) with the same regimen combined with either cladribine 5 mg/m² on days 1-5 (DAC) or fludarabine 25 mg/m² on days 1-5 (DAF). Inform consent was obtained. The goal of the current analysis was to evaluate if the addition of purine analogue affects outcome in a cohort of patients with high-risk karyotype, as defined according to SWAG criteria. Among 652 patients with newly diagnosed AML included in the PALG DAC vs. DAF vs. DA study, 111 presented with unfavorable karyotype. The median age was 50 (19-60) years. 35 patients were randomly assigned to DA arm, 35 to DAC, and 41 do DAF. In the respective study groups the rate of CR was 37%, 60%, and 54% (DA vs. DAC+DAF; p=0.05). Among CR patients 41% received consolidation followed by either HLA-matched related or unrelated donor (DA, 23%, DAC 48%, DAF 45%). With the median follow-up of 2.8 years the 3-years probability of the overall survival was 20% for DA arm, 56% for DAC and 57% for DAF (DA vs. DAC+DAF; p=0.01) (see: figure). Leukemia-free survival equaled 45%, 31% and 56%, respectively (p=NS). No significant differences could be demonstrated with regard to remission duration and non-relapse mortality. All three regimens were characterized by comparable hematological and non-hematological toxicity. We conclude that the addition of purine analogue either cladribine or fludarabine to standard induction regimen based on daunorubicin combined with AraC is associated with improved survival of patients with newly diagnosed AML and high-risk karyotype. The advantage is mainly a consequence of increased rate of complete remission.

**Background.** Older patients with AML in first relapse are frequently targeted for new drug evaluation. There are, however, few published data on their optimal management and current outcome. Aim. We performed an analysis of 393 patients aged 60y+ (median, 64y) with AML in first relapse after prospective inclusion in ALFA-9801/9803 trials (232/161). Patients. At relapse, median CR1 duration was 9 months (1-69); median WBC was 3.4 x 10^9/L; ECOG-PS was 2+ in 28% of the patients. Only 12 patients relapsed after HSCT. Diagnosis cytogenetics was: CBF, 16 (4%); intermediate, 240 (61%) including 173 normal karyotype (NK); adverse, 76 (19%); and not done 61 (16%). Twenty-nine of the 174 patients tested at diagnosis had FLT3-ITD (17%), while incidences of NPM1, double CEBPA, IDH1, IDH2, or WT1 mutation were 24%, 2%, 9%, 10%, and 2%, respectively. Among 86 NK-AML patients tested, 24 had a favorable genotype (NPM1 or double CEBPA without FLT3-ITD). Results. By physician choice, salvage was intensive chemotherapy (ICTx) in 209 (53%), low-dose cytarabine (LDAC) in 48 (12%), best supportive care (BSC) in 124 (32%), and unknown in 12 (3%) patients. Overall, CR2 rate was 30% with a significant impact of CR2 on post-relapse survival (median PRS, 16 vs 4 months; P<0.001). Duration of CR1 (<6m, 29%; 6-12m, 37%; >12m 34%) strongly influenced CR2 rate (12 vs 32 vs 47%; P<0.001) and PRS (4 vs 7 vs 11 years). 35 patients were randomly assigned to DA arm, 35 to DAC, and 41 do DAF. In the respective study groups the rate of CR was 37%, 60%, and 54% (DA vs. DAC+DAF; p=0.05). Among CR patients 41% received consolidation followed by either HLA-matched related or unrelated donor (DA, 23%, DAC 48%, DAF 45%). With the median follow-up of 2.8 years the 3-years probability of the overall survival was 20% for DA arm, 56% for DAC and 57% for DAF (DA vs. DAC+DAF; p=0.01) (see: figure). Leukemia-free survival equaled 45%, 31% and 56%, respectively (p=NS). No significant differences could be demonstrated with regard to remission duration and non-relapse mortality. All three regimens were characterized by comparable hematological and non-hematological toxicity. We conclude that the addition of purine analogue either cladribine or fludarabine to standard induction regimen based on daunorubicin combined with AraC is associated with improved survival of patients with newly diagnosed AML and high-risk karyotype. The advantage is mainly a consequence of increased rate of complete remission.
months, P<0.001). As also expected, patients with CBF-AML had higher CR2 rate (69 vs 27%, P<0.001) and longer PRS (16 vs 6 months, P=0.02). They were not included in subsequent analyses. In patients tested, FLT3-ITD predicted shorter PRS (8 vs 7 months, P=0.03). Patients with NK-AML and favorable genotype had similar CR2 rate and PRS than other patients with normal, or intermediate, or even unfavorable karyotype. In multivariate analysis, CR1<12m (HR, 1.8; P=0.001), ECOG-PS 2+ (HR, 2.0; P<0.001), age (P=0.001), and WBC (P=0.08) predicted shorter PRS, while adverse cytogenetics and FLT3-ITD did not. Patients who received ICTx displayed higher CR2 rate (52 vs 4%, P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC patients, without significant difference in PRS between LDAC and BSC (5 vs 3 months). When ICTx vs LDAC/BSC was added in the multivariate model, ICTx (HR, 0.5; P<0.001), CR1 <12m (HR, 1.7; P<0.001), ECOG-PS 2+ (HR, 1.8; P<0.001), and WBC (P=0.006) but not age remained significant, suggesting younger age was an important decision criteria for ICTx. To further evaluate the role of intensive salvage, we performed a one-to-one matching based on the propensity score for ICTx vs LDAC/BSC calculated with the following variables: age, ECOG-PS, WBC, CR1 duration, prior HSCT, and intermediate versus adverse cytogenetics. In 120 matched-pairs, ICTx was strongly associated with longer PRS (8.1 vs 4.2 months; HR, 0.50; P<0.001) and ECOG-PS 2+ (HR, 2.0; P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC. Patients who received ICTx displayed higher CR2 rate (52 vs 4%, P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC patients, without significant difference in PRS between LDAC and BSC (5 vs 3 months). When ICTx vs LDAC/BSC was added in the multivariate model, ICTx (HR, 0.5; P<0.001), CR1 <12m (HR, 1.7; P<0.001), ECOG-PS 2+ (HR, 1.8; P<0.001), and WBC (P=0.006) but not age remained significant, suggesting younger age was an important decision criteria for ICTx. To further evaluate the role of intensive salvage, we performed a one-to-one matching based on the propensity score for ICTx vs LDAC/BSC calculated with the following variables: age, ECOG-PS, WBC, CR1 duration, prior HSCT, and intermediate versus adverse cytogenetics. In 120 matched-pairs, ICTx was strongly associated with longer PRS (8.1 vs 4.2 months; HR, 0.50; P<0.001) and ECOG-PS 2+ (HR, 2.0; P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC. Patients who received ICTx displayed higher CR2 rate (52 vs 4%, P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC patients, without significant difference in PRS between LDAC and BSC (5 vs 3 months). When ICTx vs LDAC/BSC was added in the multivariate model, ICTx (HR, 0.5; P<0.001), CR1 <12m (HR, 1.7; P<0.001), ECOG-PS 2+ (HR, 1.8; P<0.001), and WBC (P=0.006) but not age remained significant, suggesting younger age was an important decision criteria for ICTx. To further evaluate the role of intensive salvage, we performed a one-to-one matching based on the propensity score for ICTx vs LDAC/BSC calculated with the following variables: age, ECOG-PS, WBC, CR1 duration, prior HSCT, and intermediate versus adverse cytogenetics. In 120 matched-pairs, ICTx was strongly associated with longer PRS (8.1 vs 4.2 months; HR, 0.50; P<0.001) and ECOG-PS 2+ (HR, 2.0; P<0.001) and longer PRS (9.5 vs 4 months, P<0.001) than LDAC/BSC.

**Conclusion.** These results confirm the value of favorable cytogenetics and CR1 duration as important prognostic factors in older patients with AML in first relapse. Awaiting new effective therapies, they also indicate that ICTx salvage should be proposed each time the patient status allows it.

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**0051.**

**CD34+ PEAK IN PERIPHERAL BLOOD DURING MOBILIZATION IS AN INDEPENDENT PROGNOSTIC FACTOR IN ACUTE MYELOID LEUKEMIA PATIENTS IN 1ST CR TREATED WITH ALLOGENIC OR AUTOLOGOUS TRANSPLANTATION**

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**Aim.** To investigate disease characteristics, treatment and outcome for adolescents and young adults treated for AML according to pediatric and adult protocols within the Nordic countries.

**Materials and Methods.** A mainly population-based retrospective survey of all patients aged 10-18 years diagnosed with AML, according to the WHO-classification, at pediatric clinics in Denmark, Finland, Iceland, Norway and Denmark 1993-2009 and all patients 15-30 years diagnosed in adult clinics in Denmark 2000-2009, Sweden 1997-2009 and a majority of the Norwegian patients diagnosed 1996-2008 were investigated. All FAB-classes were included. Secondary leukemias and patients with Down syndrome were excluded.

**Results.** 166 patients aged 10-18 years (median 13 years) with de novo AML treated according to Nordic AML 1993 and 2004 protocols were compared with 253 patients 15-30 years (median 24 years) treated in adult centres according to different national or regional guidelines. The incidence of de novo AML 2000-2009 was 5.5 per million inhabitants in the age group 10-14 years, 5.9 in 15-18 years old, and 6.2 in 19-30 years old in Sweden, the corresponding figures for Denmark were 3.9, 7.7 and 7.9, respectively. The disease characteristics differed regarding the frequency of acute
promyelocytic leukemia (APL) and cytogenetic features between the cohorts. AML in the adult cohort, 19.8% of adult leukemias compared to 5.4% in the pediatric cohort. Core binding factor (CBF) leukemia was more common in the pediatric cohort 21% versus 14.2 % in the adults. Total favorable cytogenetics (defined as t(15;17), inv(16), and t(8;21)) were thus more frequent in the adult cohort with 33.2% versus 26.5%. The MLL-rearrangements, t(9;11) and 11q23, were more frequent in the adult cohort, 9.6% than in the adult cohort with 6.0%. Overall survival (OS) at 5 years was similar for the pediatric and adult cohorts, 60.4% (52.2-67.7%) vs. 64.6% (58.1-70.3%). OS at 5 years excluding APL-patients was almost identical, 60.3% (51.9-68.7%) vs. 59.9% (52.4-66.5%). OS at 5 years was better, but not statistically significant, for female than for male AML-patients in both cohorts; in the pediatric cohort OS was 65.3% (59.0-75.5%) vs. 56.8% (45.8-66.4%) and in the adult cohort, 70.6% (61.6-77.9%) vs. 58.2% (48.6-66.7%). Conclusion. Differences in disease biology with less APL, more CBF-leukemia and more MLL-leukemia in the pediatric patients were found. No difference in outcome for AML patients aged 10-30 years, treated either in pediatric protocols or adult national/regional guidelines, was found. Age could not be shown to be an independent prognostic marker in this mainly population-based material from the Nordic countries.

**0053**

THE ADDITION OF LOW DOSE GEMTUZUMAB OZOGAMICIN TO INDUCTION, CONSOLIDATION AND MAINTENANCE THERAPY OF ELDERLY PATIENTS WITH NON M3 AML: UPDATE OF GENOA EXPERIENCE AND ANALYSIS OF PROGNOSTIC FACTORS


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**Background.** Elderly AML patients and patients with AML evolved from MDS or therapy related sAML display a very poor prognosis. We showed that adding low dose gemtuzumab ozogamicin (GO) to fludarabine, Ara-C and anthracycline (MY-FLAIS) may improve DFS and OS without increasing toxicity compared to a historical cohort of patients treated with fludarabine, Ara-C and idarubicin (FLAII), (Clavio 2007). The aim was to understand our experience and reviewed the prognostic impact of cytogenetics and molecular biology (mutations of FLT3 and NPM genes and expression of WT1 and BAALC genes) at diagnosis. Patients and Methods. Eighty-five consecutive CD33+ AML patients (≥ 60 years) were treated between May 2004 and June 2010. The median age was 60 years (60-85). The karyotype was unfavourable in 21 patients (25%), intermediate in 63 (74%) and favourable in 1 (1%). Twenty-eight patients had secondary AML (33%). The induction therapy consisted of Fludarabe 25 mg /sqm, Ara-C 1 g /sqm, idarubicin 5 mg / sqm, all for 3 days, followed by GO 3 mg / sqm at day 4. Complete responders received the same regimen as consolidation therapy. Twelve-monthly (1x) and 18-monthly (2x) doses of GO were administered (GO 5 mg /sqm) with chemotherapy (Cytarabine, 1 g /sqm every 12 hours for three times), every 3 months. Results. Neutrophil (N > 0.5 x 109 / l) and platelet (Pt > 25 x 109 / l) recovery required a median of 15 days (range 10-26) and 16 days (range 7-30) from the end of therapy, respectively. There were four early deaths during induction (5%). Twenty-six major infectious complications were recorded (sepsis in 15 patients, pneumonia in 3, aspergillosis in 5, other infections in 2). Non-haematological toxicity was very mild. In particular, none of the patients experienced grade 3 or 4 hepatic toxicity. Forty-seven patients (55%) achieved a complete remission (CR) (seven after a second induction course). No clinical or molecular parameters were significantly associated with CR rate, whereas complete remission rate was 64% and 28% in good-intermediate / poor karyotype patients, respectively (p 0.03). Median duration of CR and OS were 9 months (range 1-70) and 12 months (range 1-72), respectively; 66-month projected DFS and OS were 12% and 18%, respectively. Cox regression analysis disclosed that good-intermediate karyotype patients had a better DFS (p=0.006) and OS (p=0.002) than those with poor risk cytogenetics. Patients with denovo AML had a better DFS compared to patients with sAML (p=0.029). Among molecular markers only BAALC expression > 1000 was predictive OS, although with borderline statistical significance (p 0.05). Conclusions. The addition of low dose GO to induction, consolidation and maintenance therapy is well tolerated and associated with a good antileukemic activity. In elderly AML patients treated with MY-FLAIS classical prognostic factors such as karyotype and clinical history (de novo vs secondary disease) appear to be more important than molecular markers.

**0054**

BLASTIC PLASMACYTOID DENDRITIC CELL LEUKEMIA: PRELIMINARY RESULTS OF A RETROSPECTIVE ITALIAN MULTICENTRIC STUDY

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**Background.** Blastic plasmacytoid dendritic cell leukemia (BPDC/CL) are rarely reported. **Aims.** To evaluate in patients (pts) with BPDC/CL the clinical features, the prognostic factors, and the efficacy of treatments. **Methods.** A multicenter retrospective cohort study was carried out between January 2005 and December 2010 in 26 Italian hematology divisions. Results. According to WHO-2008 classification, a total of 42 evaluable cases of BPDC/CL were collected (M/F 31/11; median age 57 y.o., range 20-81). At diagnosis the median values of hemoglobin, white blood cell count, and platelet counts were 5.2 g/dl (range 4.5-16.6), 2.1 x 10^9/L (range 0.5-34.1), and 27 x 10^9/L (range 1-428), respectively. The median bone marrow infiltration was 90%. In 92% of cases constitutional symptoms (fever, fatigue, weight loss) were present, while only 3% were asymptomatic; 83 pts (78%) had peculiar skin lesions, while lymph node and/or spleen involvements were documented in 22 (52%). Extramedullary disease was described in 5 cases and included: skin and bone (4 cases), and lymph node (1 case). Complete remission was achieved in 5 of 30 pts (17%), with a median of 17 months (range 2-170) of follow-up (FU). In 22 pts (52%) cytogenetic study was performed, revealing an unfavourable karyotype in 9 (40%), in 6 characterized by complex chromosome aberrations. Molecular biology studies were performed in 10 pts, showing in 8 the FLT-3 ITD mutation. Forty pts received an acute leukemia-like induction therapy (2 died early, receiving only palliative treatments), that consisted of anthracycline and cytarabine or equivalent (AML-like regimen) in 29 (72%), and of dexamethasone, vincristine, cyclophosphamide, metotrexate (ALL-like regimen) in 11 (28%); 1 pt received in addition radiotherapy for cutaneous localizations. Nine pts (22%) underwent allo-HSCT as part of first-line (2) or salvage treatment (7). A complete (CR) or partial remission (PR) after induction therapy was achieved in 19 (45%) and 6 pts (14%) respectively (overall response rate 59%). It was registered 8 CR and 2 PR after ALL-like regimen, and 11 CR e 4 PR after AML-like regimen, with a significant advantage for ALL-like chemotherapy (p=0.04). The median overall survival (OS) was 8 months (range 0.2-60); 22 months (range 2-31) and 8 months (range 0.2-60) in pts received ALL-like regimen and AML-like regimen respectively (p=0.05). In HSCT-pts the median OS were 31 months (range 3-60), with a significant advantage with respect to non-transplanted pts (median 6 months, range 0.2-26, p=0.004). In pts obtaining a complete remission, the median disease free survival was 9 months (range 0.2-60), among them 18 pts relapsed, after a median time to diagnosis of 4 months (range 1-9). As for the transplanted pts, was still alive in CR at last follow up. The overall mortality rate was 43% at 6 months and 73% at 12 months. Summary/Conclusions. BPDC/CL is a
rare hematopoietic neoplasia, preferentially involving skin, bone marrow, and lymph nodes, characterized by a poor prognosis. Initial response to acute leukemia-like chemotherapy is good, but relapse occurred very rapidly after a median time of only 4 months; allo-HSCT performed in first remission may lead to long-term survivor and disease control in selected cases, but more data are needed to confirm these preliminary results.

0055
THE EXPRESSION PATTERN OF MICRORNAs (MiRNA) MIGHT ADD RELEVANT PROGNOSTIC INFORMATION TO MOLECULAR CATEGORIZATION OF INTERMEDIATE RISK CYTOGENETIC ACUTE MYELOID LEUKEMIA
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Background. The prognosis of AML patients within the intermediate cyogenetic category is mainly determined by the mutational status of some relevant genes, such as NPM1 mutations (NPMmut), or biallelic CEBPA mutations (CEBPAmut), associated with a favorable outcome, and with the presence of FLT3 internal tandem duplication (FLT3-ITD), which correlates with an adverse prognosis. Nonetheless, additional biological features such as miRNA expression pattern might contribute to refine prognosis and guide therapy in this setting. Aim. To investigate whether miRNA expression is associated with molecular characteristics and clinical outcome in intermediate-risk AML patients (IR-AML).

Methods. We have analyzed samples from 85 IR-AML patients (median age, 52 [range, 18-71]; 52% males) who received intensive therapy for AML, including 7 with biallelic mutations. The expression of 670 mature miRNAs was analyzed by multiplex Real Time PCR multiplex permutations (class comparisons analysis, p<0.001) revealed that 247 miRNAs showed significant differences in expression levels between the different subcategories of IR-AML patients. Results. Among the different subgroups identified, miR-196b (p=0.056; HR=7.27, CI: 0.95-55.6), miR-9 (p=0.03; HR=14.2, CI: 0.18-114.7), and increased level of let-7a* (p=0.026; HR=5.1, 95% CI: 1.21-21.5) and miR-196b (p=0.056; HR=7.27, CI: 0.95-55.6). Concerning risk of relapse, the absence of NPMmut, FLT3-ITD and increasing leukocyte count were associated with a higher RI. Remarkably, decreased miR-9-3p expression (p=0.011; HR=3.3, 95% CI: 1.8-6.2) and miR-15a (p=0.02; HR=4.2, 95% CI: 1.2-14.2), together with higher levels of miR-23a* (p=0.001; HR=6.2, 95% CI: 2.61-14.7) were independently associated with a higher relapse risk. Of note, a decreased miR-9-3p level retained its adverse prognosis value in the subgroup of patients without favorable molecular markers (i.e., wild-type NPM1 and CEBPA/ or FLT3-ITD; p=0.001, see figure). On the contrary, let-7a* levels segregated subgroups of patients in the category of favorable cytogenotype (i.e., mutated NPM1 without FLT3-ITD) (p=0.07). Conclusions. In this series of patients of intermediate-risk cytogenetic AML, measurement of expression levels of several miRNAs such as miR-9-3p, miR-15a, let-7a* or miR-23a* showed independent prognostic value, and might contribute to predict the outcome within specific molecular subgroups. Nonetheless, confirmation of the prognostic impact of these miRNAs and investigation of possible underlying mechanisms account for this effect require further studies.

0056
EFFICACY OF HISTAMINE DIHYDROCHLORIDE AND INTERLEUKIN-2 IN MORPHOLOGIC SUBTYPES OF ACUTE MYELOID LEUKEMIA
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Background. The combination of histamine dihydrochloride (HDC) and low-dose interleukin-2 (IL-2) was recently approved in Europe as relapse-preventive immunotherapy in acute myeloid leukemia (AML). HDC has been shown to block the formation of immunosuppressive oxygen radicals in normal myeloid cells that express a radical-generating NADPH oxidase and histamine H2 receptors (H2R). HDC thus preserves anti-leukemic functions of normal cells and cytotoxic T cells. Aims. 1) To investigate the expression of NADPH oxidase components and H2R on leukemic cells in morphologic subtypes of newly diagnosed AML. 2) To determine the outcome of HDC/IL-2 immunotherapy in morphologic subtypes of AML. Methods. Flow cytometry was employed to screen freshly recovered leukemic cells from the blood or bone marrow of patients with morphologic subtypes of newly diagnosed AML and to monitor the expression of H2Rs and the NADPH oxidase component gp91phox. Leukemia-free survival (LFS) was determined in patients receiving HDC/IL-2 or no treatment (control) in a phase III trial as described in detail elsewhere (Brune et al., Blood 108:85-96). Results. H2R and gp91phox were commonly co-expressed in a fraction (10-60%) of malignant cells recovered from patients with AML of M4 and M5 FAB classes. In contrast, H2R or gp91phox were undetectable on leukemic cells from patients with FAB/M2 AML. In line with these findings, a post-hoc efficacy analysis of the phase III trial using HDC/IL-2 in AML revealed that patients with FAB/M2 AML in first CR did not benefit from HDC/IL-2 immunotherapy, even when the analysis was carried out in patients with a strong overall benefit of HDC/IL-2 (age <60, p>0.7, HR=1.14, n=41). In contrast, corresponding HDC/IL-2-treated patients with non-M2 AML displayed significantly higher LFS than control patients (52 vs. 25 %, LFS at 3 years, p<0.001, HR=0.43, CI: 0.27-0.69, n=113). The LFS benefit was pronounced in patients with M4 or M5 AML (63 vs. 27 % at 3 years, p<0.01, HR=0.37, CI: 0.18-0.75, n=58). Summary and Conclusions. These findings imply that HDC/IL-2 immunotherapy predominantly targets monocytic forms of AML, which may be explained by co-expression of histamine receptors and gp91phox on leukemic cells of the monocytic lineage.

0057
RASGRF1/APTX RATIO STRONGLY CORRELATES WITH CLINICAL RESPONSE AND SURVIVAL IN AML PATIENTS TREATED WITH TIPIFARNIB-BORTEZOMIB COMBINATION
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Background. We conducted a phase I-II study aiming to assess efficacy and toxicity of tipifarnib-bortezomib treatment in elderly AML patients. RASGRF1/APTX genetic ratio, which is associated with treatment response in patients treated with tipifarnib alone, was tested.
Methods. Eighty patients were enrolled with secondary-AML: 42 had high risk cytogenetics; 42 were previously untreated. Seventy-five patients were treated. Results. Nine patients achieved complete remission (CR), 1 patient obtained a partial response (PR) and in 2 cases an hematological improve-
ment (HI) was documented for an overall response rate (ORR) of 19%. Me-
dian time to response was 112 days, corresponding to 4 cycles (range 2-14).
Marrow response (CR+PR) was significantly associated with overall survival (OS) (p<0.0001). RASGRP1/APTX was evaluated before treatment, at initia-
tion on bone marrow (BM), peripheral blood (PB) or both. The median RASGRF/APTX value on BM was 15.3 (15-18.9) in responder patients and 2.2 (0.5-25.9) in non responders, respectively (p=0.0006). Its median value on PB was 31.6 (19.3-35.5) in responders and 6.4 (0.5-27.1) in non respon-
ders, respectively (p<0.0001). Interestingly, no marrow response was re-
corded in patients with marrow RASGRF/APTX ratio <8, while the re-
sponse rate was 43% (how many were CR) in patients with RASGRF/APTX >8 (p=0.0001). Finally, RASGRF/APTX levels signifi-
cantly correlated with OS (p=0.001) with a median OS of 490 days and 162 days in patients with RASGRF/APTX >8 and <8 respectively. Conclu-
sion. We confirm that the clinical efficacy of the combination of tiaplabam and bortezomib was evident. We confirmed that the RASGRF/APTX BM or PB level is an effective predictor of response and survival and our own study provided the response of such patients to tiaplabam, alone.

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0058

DNMT3A EXON 23 MUTATIONS IN ACUTE MYELOID LEUKEMIA: A SINGLE CENTER EXPERIENCE

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Background. It has been recently shown that DNMT3A mutations are recurrent in patients with intermediate-risk cytogenetic AML and are associated with a poor outcome (Ley T et al., NEJM, 2010). We have assessed the clinical characteristics and prognostic impact of DNMT3A exon 23 mutations (DNMT3Am) in a series of 210 patients treated at the Hematology department of Toulouse, France. Aims. To assess the prognostic impact of DNMT3Am in AML patients treated with intensive chemotherapy. Methods. We used a real-time High Res-
olution Melting (HRM) polymerase chain reaction (PCR) to identify a somatic mutation in the exon 23 of DNMT3A. Positive cases detected by the HRM analysis were sequenced to confirm the mutation. FLT3 and NPM1 mutations were assessed multiplex PCR and capillary electrophoresis. We used samples from 210 AML patients (65y or younger) stored at the HIMIP cell bank in Toulouse University Hos-
pital. Patients were treated by a “+7+”induction chemotherapy (be-
tween 01/2000 and 12/2009). Responding patients with HLA-identical sibling were allocated to allogeneic stem cell transplantation (allo-
SCT), others to autologous-SCT or high dose Ara-C. Study population resulted in concordant results between flow cytometry and RNA hybridization. Cor-
relation coefficients were calculated for the most relevant 18 antigens using mRNA values (number of molecules/sample) and values ob-
tained from flow cytometry (% of positive cells/sample, in compar-
ison to isotype controls).

Methodology Coefficients for nCounter and flow cytometry results calculated, based on >1600 comparisons. Highly significant values between 0.4 and 0.9 were found for the 18 antigens tested. A second correlation analysis performed on a sample basis and specifically on the blast popu-
lation resulted in concordant results between flow cytometry and nCounter in 54 - 100% of the antigens tested, depending on the hybridization.

Summary / Conclusions. The diagnosis of malignant hematological diseases has become increasingly complex during the last decades. It is based on the interpretation of results from different laboratory analyses, which range from microscopy to gene expression profiling. Recently, a new method for the analysis of mRNA phenotypes has been developed, the nCounter technology (Nanostring® Technologies), which allows for the quantification of hundreds of mRNA molecules in biological samples. We evaluated this technique in a Swiss multi-center study on samples from acute leukemia (AL) patients. Material and Methods. Peripheral blood (PB) and bone marrow (BM) samples were obtained from healthy controls (PB, 11 BM) and from patients with AL and various other hematological diseases (58 AML, 8 ALL, 2 CML, 3 CLL, 3 MDS), and referred to the Geneva Haematology Service. 43 AL samples were obtained from other Swiss Centers. For each patient a hematological work-up was performed, including a detailed flow cy-

mometric analysis. Leftover material was used for the quantification of a set of 40 different mRNAs using the nCounter technology. Initially, 100 ng of purified RNA from each sample were hybridized with probes specific for the corresponding 85 surface antigens, and every specific hybridization product was then quantified. Subsequently, we explored one of the many advantages of the nCounter technology, namely the fact that crude RNA without any enzymatic treatment is hybridized on nArray and thus used total cell lysates in the hybridization. Cor-
relation coefficients were calculated for the most relevant 18 antigens using mRNA values (number of molecules/sample) and values ob-
tained from flow cytometry (% of positive cells/sample, in comparison to isotype controls). Results. mRNA and protein profiles were es-
tablished for normal PB and BM samples. Relative signal intensities and expression patterns of the various surface antigens were similar to those described in the literature and found in previously performed Affymetrix microarray analyses. Acute leukemia samples were ana-
alyzed with this validated set of antigens and the Spearman Correla-
tion Coefficients for nCounter and flow cytometry results calculated, based on >1600 comparisons. Highly significant values between 0.4 and 0.9 were found for the 18 antigens tested. A second correlation analysis performed on a sample basis and specifically on the blast popu-
lation resulted in concordant results between flow cytometry and nCounter in 54 - 100% of the antigens tested, depending on the number of blasts present and the type of leukemia (AML versus ALL). Discordant results could be attributed mainly to samples with mRNA expression but lacking expression of the corresponding protein on the cell surface, and to myeloid antigens with an already high mRNA and protein expression in normal non-leukemic samples. Conclusion. The nCounter allows the fast and easy establishment of a mRNA profile from hematological samples. Correlation between the values of mRNA quantity obtained by this technique and protein expression, as measured by flow cytometry, is excellent for most of the antigens tested. Potential advantages of this new technology in the diagnosis of
AL, especially of rare AL cases and in MPAL (mixed phenotype acute leukemia), will be discussed.

**0060**

**COMPARATIVE ANALYSES OF GENETIC ALTERATIONS IN PAIRED SAMPLES FROM 118 ADULT AML PATIENTS AT DIAGNOSIS AND RELAPSE**

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**Background.** Acute myeloid leukemia (AML) represents a clinically and biologically heterogeneous malignancy with uncontrolled proliferation of hematopoietic precursors. Although the majority (70-80%) of AML patients achieve a complete remission (CR) after induction chemotherapy, about a half of these patients relapse with a gravid prognosis. However, the genetic alterations at relapse have not been extensively studied. **Aims.** In this study, we aimed to compare the genetic aberrations between AML at diagnosis and at relapse to determine their roles in AML development and disease progression. **Methods and Materials.** Comparison of genetic alterations, including Class I mutations (missense mutations of N-RAS, K-RAS, PTPN11, JAK-2, KIT tyrosine kinase domain of FLT3 (FLT3/ITD) and internal tandem duplication of FLT3 (FLT3/ITD) and Class II mutations, such as mutations of CEBPA, AML1/RUNX1, and partial tandem duplication (FTD) of MLL as well as NPM1 and WT1 mutations, at relapse with those at diagnosis was performed in paired samples from 118 patients with de novo AML. **Results.** One hundred and one (85.6%) patients had at least one molecular gene mutation and 56 (47.5%) had at least two mutations at diagnosis. At relapse, 85 (72%) out of the 118 patients had at least one molecular gene mutation, and 40 patients (33.9%) had two or more mutations. Among the patients with gene mutations, concurrent Class I and Class II or Class I and NPM1 mutations, which behave more like Class II mutations, could be demonstrated in 46.5% (47/101) at diagnosis and in 38.8% (38/98) at relapse. Mutational shifts occurred in 55 patients (46.6%). Class I mutations, which activate signal transduction, were lost more frequently (around 50%) at relapse than Class II mutations, which perturb transcription factors. Genetic evolution with acquisition of novel mutations at relapse were identified in 14 individuals (11.9%), all involving Class I or WT1 mutations, but not Class II or NPM1 mutation. The patients with genetic alterations at relapse had poorer overall survival. Stepwise Cox proportional hazards modeling showed that presence of FLT3/ITD, WT1, KIT or MLL/PTD at relapse were independent poor prognostic factors. **Conclusions.** The findings from this study support the two-hit theory not only in the development but also in the progression of AML. The fact that Class I mutations are frequently lost and can be acquired at relapse, in contrast to Class II mutations or NPM1 mutation, suggests that Class II and NPM1 mutations act as the second hit. In addition, genetic alterations at relapse can predict the clinical outcome.

**0061**

**GERM-LINE MUTATIONS IN THE DNA DAMAGE RESPONSE GENES BRC1, BARD1 AND TP53 IN PATIENTS WITH THERAPY-RELATED MYELOID NEOPLASMS**

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**Background.** Therapy-related myeloid neoplasms (t-MNs) are severe long-term sequelae of cytotoxic treatments for a primary, often malignant disorder accounting for 10% of all MDS/AML cases. Increasing evidence from animal and human studies pinpointed a strong influence of genetic predisposition in the pathogenesis of these secondary malignancies. **Aims.** Cases with t-MNs frequently exhibit a remarkable family history of cancer. We therefore hypothesized that cancer predisposition syndromes are prevalent in this cohort of patients and aimed at identifying germ-line mutations in respective candidate genes. **Methods.** A “nuclear pedigree” consisting of all first- and second-degree relatives was obtained from 51 adult and pediatric index patients with t-MNs and evaluated for cancer predisposition syndromes according to established criteria. In conspicuous cases, genomic DNA from cultured skin fibroblasts of t-MN patients was analyzed for deleterious germ-line mutations by PCR and direct sequencing as well as for large genomic rearrangements by MLPA. Mutations and genetic variants were classified according to public databases. Deleterious heterozygous germ-line mutations were further assessed for loss of the wildtype allele in CD54+ sorted leukemic cells by PCR, direct sequencing and SNP/CNV microarrays (Affymetrix GeneChip Human Mapping SNP 6.0 arrays), respectively. **Results.** Twenty-five of 51 (49%) patients with t-MNs had a hematological malignancy and 26 (51%) a solid tumor as primary disease. Non-Hodgkin’s lymphoma (29%), breast cancer (BC) (25%) and sarcoma (8%) were the most frequent primary neoplasms found in more than 60% of all patients. Six pedigrees indicated a hereditary breast and ovarian cancer syndrome and ten a Li-Fraumeni (LF) or LF-like syndrome initiating a search for BRCA1, BRCA2, BARD1 and TP53 germ-line mutations, respectively. Deleterious, heterozygous germ-line mutations were found in 5/51 (9.8%) individuals: two in BRCA1 (c.5251C>T, p.R1751*; c.3112G>T, p.E1038*), one in BARD1 (c.1676G>C, p.C557S) and two in TP53 (c.1146delA, p.K382fs*40; c.849-852insGCGGC, p.R283fs*22). The TP53 mutations have not been described before. Both, BRCA1 c.3112G>T and TP53 c.849-852insGCGGC germ-line mutations showed homozygosity in sorted CD54+ leukemic cells and SNP/CNV microarray analysis revealed loss of the wildtype allele in either cases. **Conclusions.** We found that approximately 5% of cancers arise in the context of hereditary cancer predisposition syndromes, deleterious germ-line mutations are found with increasing prevalence in this cohort of t-MN patients. Preliminary data indicate that these mutations contribute to therapy-related leukemogenesis. Furthermore, our data may have clinical implications with respect to genetic counseling of these patients and their relatives.

**0062**

**EXPRESSION PATTERN OF WT1 ISOFORMS IN PATIENTS WITH CHILDHOOD AML**

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**Background.** Wilms’ tumour gene 1 (WT1) is overexpressed in a large proportion of acute leukemias and other haematological malignancies. It has been demonstrated that WT1 protein is produced in more than 50% of cases of acute myeloid leukemia (AML). However, only limited information on WT1 isoforms has been published so far and the relevance of their expression pattern remains unclear. **Aims.** We determined the expression pattern of four WT1 isoforms characterized by the presence or absence of exon 5 and KTS insert (A5+/−, B(+/−), C(+/−), D(+/−)) in childhood AML using a specific qPCR system. **Methods.** We designed a unique qPCR system for detection and quantification of 4 major WT1 isoforms and verified the sensitivity, specificity and reproducibility of the results in extensive testing. **Results.** We found an excellent correlation between the total WT1 expression and the sum of WT1 isoforms levels (r=0.973, p<0.0001). Analysis showed diverse expression patterns of WT1 isoforms in the particular leukemic cell lines (p<0.0001). **Conclusions.** We identified a similar pattern of WT1 isoforms in cell lines with WT1 promoter rearrangement independent of WT1 isoform expression (e.g. MV4;11 and lymphoid - MV4;11 and lymphoid - RS4;11). The analysis of healthy controls (bone marrow or peripheral stem cells) failed to define the physiological ratio of WT1 isoforms. Very low levels of total WT1 in these samples precluded detection of WT1 isoforms since we reached the limit of the sensitivity of qPCR method (as defined by repeated dilution experiments). For the same reason of very low total WT1 levels, 11 patients (mostly AML M5) were excluded from the further analyses. Moreover, in all analysed patient diagnostic samples (N=53) a uniform WT1 isoforms expression pattern was present (A=C=B=D) with
Exon3[+] variants overexpression. We observed a trend to a higher level of isoform C in M1 and M3 patients and PML/RARA patients (Kruksal-Wallis p=0.0307 and p=0.0063, respectively). Conclusion. This is the first report of the analysis of WT1 isoforms expression pattern in childhood AML using a unique qPCR method for the detection of WT1 variants. Although the ratio of WT1 isoforms may vary in different tissues and cell lines, our data suggest that the WT1 isoforms expression patterns are useful in pediatric AML with predominate expression of Exon3[+] isoforms and possible variations in isoform C levels depending on the morphological and genetic characteristics. Careful pre-analytical testing of the detection system parameters suggests the technical limitations in detection of WT1 isoforms in samples with very low levels. Therefore, the previously reported differences in WT1 isoforms ratio between normal bone marrow and AML samples should be interpreted with caution.


**0063 LEVEL OF MINIMAL RESIDUAL DISEASE (MRD) AND WHITE BLOOD CELL COUNT (WBCC) DISCRIMINATE CATEGORIES OF PATIENTS WITH DIFFERENT OUTCOME AMONG ADULTS WITH FAVOURABLE-RISK ACUTE MYELOID LEUKEMIA**

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**Background.** Core binding factor acute myeloid leukemia (CBF-AML) and AML with mutated Nucleophosmin (NPM) without FLT3-ITD mutation are currently regarded as favourable-risk AML. Recent findings suggest a biological and prognostic heterogeneity of this AML subgroup, particularly of CBF-AML. Aims. The aim of our study was to assess whether MRD detection was able to identify patients with increased risk of relapse. **Material and Methods.** MRD was determined by multiparametric flow cytometry (MFC) on bone marrow (BM) samples collected at the end of consolidation therapy. The threshold for MRD negativity was set below the level of 3.5x10^{-4} residual leukemic cells. We evaluated 59 patients with de novo AML, enrolled sequentially in AML10/AML12 (n=48) and AML17 (n=11) EORTC/GIMEMA randomized trials between 1995 and 2007. In AML10/AML12 protocols, patients aged <60 years received induction treatment that combined standard or high dose of cytarabine (ARA-C), according to randomization, etoposide, and anthracycline. As consolidation, all patients received intermediate dose of ARA-C and antracycline. Thereafter, those with an HLA-compatible sibling were allografted. Patients without a compatible donor underwent autologous stem cell transplantation (AuSCT). Patients aged >60 years (AML17) received mitoxantrone, ARA-C, and etoposide and 2 cycles of a consolidation program consisting of idarubicin, cytarabine, and etoposide. All patients were randomized before induction, to receive or not, gentuzumab ozogamicin, repeated on day 1 of each consolidation cycle. Median age was 48 years (range 24-69 years) and 80% of patients were older than 60 years (AML17). 23 females and 46 (81%) had white blood cell count (WBCC) <5 x 10^9/L. Twenty-nine CBF-AML [21 with t(8;21) and 8 with inv(16)] and 30 NPM-AML were evaluated. Overall 24 patients (41%) relapsed, 3 NPM-AML patients experienced an early relapse after consolidation therapy. After first consolidation, 21 patients underwent AuSCT, 15 AlloSCT and 23 did not receive any transplant procedure: 11 because of age, the remunerates due to refusal or medical reasons (2 of 12 were consolidated with high dose ARA-C). Results. MRD positive status after consolidation (MRDpos) and WBCC ≤5 x 10^9/L were significantly associated to relapse (p=0.017 and 0.0001, respectively). At 4 years, DFS for patients MRDneg vs MRDpos and with WBCC ≤5 x 10^9/L was 70% vs 44% and 59% vs 21% (p=0.018 and 0.011, respectively). Accordingly, cumulative incidence of relapse (CIR) at 4 years for patients MRDneg vs MRDpos and with WBCC ≤5 x 10^9/L was 21% vs 55% and 35% vs 85% (p=0.005 and <0.001, respectively). Therefore, we identified 3 different groups of patients based on the combination of MRD status after consolidation and WBCC. At 4 years, DFS for MRDneg/WBCC≤5 x 10^9/L, MRDpos/WBCC≤5 x 10^9/L and MRDpos/WBCC>5 x 10^9/L was 77%, 49% and 15%, respectively (p=0.001) and CIR at 4 years was 12%, 47% and 88% (p=0.001). Conclusion. Combined evaluation of WBCC and post-consolidation MRD status enables identification of patients at higher risk of relapse in spite of a favorable risk genetics/cytogenetics profile for whom intensification by AlloSCT should be considered.

**0064 TREATMENT OF MOLECULAR RELAPSE IN PATIENTS WITH ACUTE MYELOID LEUKEMIA**

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**Background.** Evidence for beneficial effect of minimal residual disease (MRD) monitoring and early intervention (=pre-emptive therapy) in non-APL acute myeloid leukemia (AML) pts. are very limited. Aims. To assess usefulness, efficacy and toxicity of various treatment regimens in the therapy of molecular relapse in non-APL AML patients. Methods. We have performed a retrospective analysis of molecular relapses in AML pts. with molecular target (RUNXI-RUNXIT1, CBFB-MYH11, MLL fusion and NPM1) and its treatments. RQ-PCR was used for the MRD monitoring. Molecular relapse was defined as confirmed reappearance of the fusion transcript or mutated gene detection or its 10-fold increase in pts. with persistent positivity and corresponding bone marrow cytology, immunophenotype and cytogenetic analysis remained negative. Results. In the study period 1/1/2008 - 31/12/2010 we have treated 30 molecular relapses in 19 pts. Median follow up was 24 months (range 8-95 months). The median time from the end of previous therapy to molecular relapse was 5.6 months (range 0.3-31 months). 23 pts. (77%) were Fulfilling the criteria after allogeneic hematopoietic stem cell transplantation - allo HSCT (30%). The overall response rate was 66% (CMoR - 53%, PmoR - 13%, SD - 13%, progression - 20%). The highest response rate was achieved with clofarabine - 88% (CMoR - 66%, PmoR - 12%, SD - 0%, progression - 22%) and conventional chemotherapy - 84% (CMoR - 80%, PmoR - 34%, SD - 16%, progression - 0%). The response rate for GO was 60% and for immunomodulation after HSCT 44%. We observed neutropenia and thrombocytopenia gr. III-IV (according to CTCAE 4.0) in 100% cases treated with clofarabine, conventional chemotherapy or GO and only in 11% and 0% respectively in patients after immunomodulation after HSCT. Severe complications occurred in 17% cases treated with conventional chemotherapy (one death for toxicity) and in 11% cases treated with clofarabine, but not in patients treated with GO or immunomodulation. 30% of patients underwent allo HSCT. New relapse during follow up period was observed in 58% with median 7 months (range 3-18 months). However only 50% (8/16) of cases that achieved CMoR after treatment of molecular relapse relapsed in follow up period; in opposite 100% (3/3) patient with only PmoR after treatment of initial relapse relapsed in follow up period. One-year disease free survival was 56%, one-year overall survival was 74%. Summary/Conclusions. Our study has shown feasibility of MRD monitoring and pre-emptive therapy strategy using molecular targets in non-APL AML pts. This approach had reasonable toxicity. Different pre-emptive treatment strategies led to response in 2/3 of molecular relapses and clofarabine led to the highest % of CMoR (66%). However, even using allo HSCT as the part of molecular relapse treatment, in 56% of responders new relapse occurred with median of 7 months.

**0065 AGE-DEPENDENT ANALYSIS OF THE PROGNOSTIC IMPACT OF MINIMAL RESIDUAL DISEASE (MRD) IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA**

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**Background.** Although the therapy of acute myeloid leukemia (AML) has witnessed remarkable progress over the last 2-3 decades in, two thirds of young adults still die of their disease. In older adults, who represent the majority of patients with AML, the results are even more unsatisfactory. In fact, while 40-50% can achieve a complete remission (CR), less than 10% are long-term survivors. Based on this, age is universally recognized as a pivotal prognosticator affecting outcome and treatment choice. In consecutive series of adult patients with de novo AML, we have repeatedly demonstrated the prognostic role of (MRD) detection, by flow cytometry. In particular, we have found that a level
of MRD ≥ 5.5x10e-4 leukemic cells at the end of consolidation is associated with a relapse rate of 70-80%. Aim: In the present study we evaluated whether the prognostic impact of MRD assessment after consolidation remained unaltered even in age-stratified (≤ 60 and > 60 years) populations of adult patients with de novo AML. Methods: For the purpose of this study, we analyzed 128 young adults (median age 40, range 18-60) and 85 elderly adults (median age 67, range 61-79). The two cohorts were well balanced in terms of frequency of FLT3-ITD and NPM1 mutated cases. A lower frequency of good-risk karyotypes was observed in elderly vs young patients (4% vs. 20%, p=0.014). Results: The frequency of MRD negative measurements was lower among elderly patients as compared to the younger ones (7% vs 13%, p=0.002). Among 48 MRD positive elderly, 40 (83%) have relapsed and 8 (17%) have not; among 7 MRD negative, 6 (57%) have relapsed and 1 (14%) have not. Overall survival (OS) and disease free survival (DFS) were significantly longer for patients who were MRD negative at the post-consolidation time-point (p=0.001 for both comparisons). On the opposite, among 86 MRD positive younger patients, 51 (59%) experienced a relapse and 35 (41%) did not whereas among 42 MRD negative, 11 (26%) have relapsed and 31 (74%) are in continuous remission (p=0.001). OS and DFS were significantly longer for patients who were MRD negative at the post-consolidation time-point (p<0.001 and p=0.002, respectively). Cumulative incidence of relapse (CIR) was significantly lower for the MRD negative group both in elderly (57% vs. 88%, p<0.001) and younger patients (30% vs. 70%, p=0.002). Conclusions: Elderly adults, below-cytometric MRD negativity defines a subgroup of patients with a CIR that is significantly lower than that of MRD positive patients (57% vs 88%, p=0.002). Nonetheless, elderly patients infrequently become MRD negative at 13% vs 33%, p=0.005 and, even when MRD negativity is obtained, the rate of relapse doubles that of the younger counterpart (57% vs 26%, p=0.005), confirming that age represents by itself a poor-risk feature in AML.

**0066**

THE IMPACT OF PRIOR HYPOMETHYLATING AGENT TREATMENT AMONG SECONDARY AML PATIENTS TREATED WITH CPX-351 OR 7+3 CHEMOTHERAPY

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Background. CPX-351 encapsulates cytarabine and daunorubicin at a 5.1 molar ratio predicted in vitro to maximize synergy. CPX-351 liposomes accumulate within bone marrow with preferential uptake by leukemia cells followed by intracellular release of drug. Multiple CRs in advanced AML patients in Phase 1 led to a randomized Phase 2 study comparing CPX-351 (85 pts) vs. standard cytarabine + daunorubicin (7+3) (41 pts) for untreated older patients with AML. CPX-351 showed improvement in CR + CRi rate, 60 day mortality, DFS and OS. Significant improvement in OS was observed among 51 (40%) patients entered with secondary AML (prior antecedent hematologic disorders (AHD): MDS, MPN, CMMoL, and treatment related AML. Previously treated patients with AHDS, particularly prior hypomethylating agent therapy for MDS, identifies a subgroup with poor outcomes. Following analyses were performed to see whether the apparent improvement in outcomes following CPX-351 treatment of secondary AML patients might extend to patients with prior hypomethylating agent treatment. Aims: To evaluate the impact of prior hypomethylating agent therapy on the outcome of AML induction treatment with CPX-351 compared to 7+3 therapy. Method: A total of 85 untreated de novo or secondary AML patients, aged 60-75, PS = 0-2, S < 0.2 mg/dL, total bilirubin < 0.2 mg/dL, ALT/AST < 3 x ULN, and LVEF > 50%, were eligible. Patients were randomized 2:1 to receive up to 2 induction and 2 consolidation courses of CPX-351 (100 µm; d1, 5, 9 min infusion, 1 unit = 1 mg cytarabine + 0.44 mg daunorubicin) or standard 7+3 treatment (cytarabine 100 mg/m2/d CI x 7d and daunorubicin 60 mg/m2 d1, 2, 3). Consolidation with hematopoietic stem cell transplantation (HSCT) was permitted. Endpoints included: CR + CRi rate (1st endpoint) and duration, EFS, aplasia rate, survival at 1-year, and death at day 30 and 60. Results. This analysis focuses on the group of secondary AML patients randomized to CPX-351 (52 pts) and 7+3 (19 pts), analyzing the impact of prior hypomethylating agent treatment. Twenty (39%) of 51 patients with secondary AML included in this study received prior hypomethylating agents. Three patients on CPX-351 and 2 on 7+3 had prior treatment with other agents and are not included in the table. Prior treatment with hypomethylating agents was associated with reduced response rate, EFS and proportion alive at 1 year. With small numbers of secondary AML patients CPX-351 treatment was associated with improved outcomes. Summary/Conclusions. Newly diagnosed secondary AML patients treated with CPX-351 demonstrated higher response (CR + CRi) rate, lower induction mortality, improved EFS, and improved survival (p=0.01) compared to 7+3 chemotherapy. Prior hypomethylating therapy diminished treatment efficacy in both arms of the study, but CPX-351 treatment was associated with improved response and survival in this poor prognosis group.

**Table 1.**

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<th>Pathology</th>
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<tr>
<td>Secondary AML</td>
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**0067**

THE PROGNOSTIC IMPACT OF GERMLINE 46/1 HAPLOTYPE OF JANUS KINASE 2 IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA


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Background. Risk stratification according to acquired genetic alterations received increasing attention in acute myeloid leukemia (AML) in recent years, although current prognostic factors are not sufficient to accurately predict outcome especially in AML with normal karyotype (NK-AML). Inherited polymorphisms are also candidates for the explanation of the heterogeneous prognosis. Germline Janus kinase 2 (JAK2) haplotype designated as 46/1 haplotype was reported to be associated with an inherited predisposition to JAK2 V617F positive and negative myeloproliferative neoplasms, and also to NK-AML. Aims: The aim of this study is to assess the prognostic impact of 46/1 haplotype on disease characteristics (age at disease onset, history of AML, morphology, associations with cytogenetic and molecular genetic alterations) and treatment outcome (complete remission and relapse rate, disease free survival [DFS] and overall survival [OS]) in AML. Methods: JAK2 rs12545867 SNP tagging 46/1 haplotype was genotyped by LightCycler technology applying melting curve analysis with the hybridization probe detection format in 176 patients with AML diagnosed consecutively in a single center. The participants signed informed consents, and the study was approved by the Institutional Ethics Committee. Results. The allele C of JAK2 rs12545867 co segregates with the 46/1 haplotype, while allele T of rs12545867 is linked to the wild type 46/1 haplotype carrier frequency was similar in de novo and in myelodysplasia- or therapy-related AML subgroups. Distribution of morphological subtypes were different between haplotype-carriers and non-carriers (p=0.047). There were considerably more FAB M2 morphological
cases within 46/1 non-carriers vs. carriers (17% (15/87 TT) vs. 6% (5/89 TC and CC), p=0.018), while myeloid mononuclear and monocytic variations were more frequent in 46/1 haplotype carriers [32% (28/87 TT) vs. 55% (49/89 TC and CC), p=0.008]. Similar distribution was observed in case of NK-AML. There was a tendency of increased 46/1 haplotype carrier frequency in NK-AML compared to AML with abnormal karyotypes [58% vs. 44%, p=0.069, OR (95%CI): 1.79 (0.98-3.26)]. JAK2 46/1 haplotype carriership was an independent adverse prognostic factor for DFS [HR (95%CI): 1.88 (1.07-3.29), p=0.028] and OS [HR (95%CI): 1.88 (1.07-3.29), p=0.027] in NK-AML. Conclusions: JAK2 46/1 haplotype may be a novel, independent unfavorable risk factor in AML.

**0068**

**MLN4924 A NOVEL INVESTIGATIONAL NEDD8-ACTIVATING ENZYME (NAE) INHIBITOR, IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND HIGH-GRADING MYELODYSPLASTIC SYNDROMES (MDS): RESULTS FROM A PHASE 1 STUDY**

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Background. NAE regulates the NEDD8 conjugation pathway and is required for the activity of cullin-RING E3 ligases (CRLs), which control degradation of substrates involved in cell-cycle regulation, signal transduction, DNA replication, and stress response, including proteins important for AML cell survival. Inhibition of NAE with MLN4924, a novel, investigational, first-in-class small molecule NAE inhibitor, results in accumulation of CRL substrates and leads to apoptosis, as demonstrated in AML preclinical models. Aims. The primary objectives of this multicenter study were to evaluate the safety and tolerability of MLN4924, and to establish the MTD and the recommended phase 2 dose of MLN4924 in patients with AML and high-grade MDS. Secondary objectives included a preliminary assessment of efficacy, and analysis of pharmacokinetics and pharmacodynamics. Methods. Patients aged ≥18 years, with ECOG performance status 0-2, who had AML or high-risk MDS and were not candidates for potentially curative therapy, received MLN4924 as a 60-minute IV infusion on days 1, 3, and 5 of a 21-day cycle for ≥12 months or until disease progression. Dose escalation from 25 mg/m² proceeded using a standard 3+3 escalation method. Informed consent was obtained. Results. Twenty-nine patients (19 males, 28 AML, 1 MDS) were treated, including 3, 4, 5, 13, and 4 at dose levels of 25, 33, 44, 59, and 75 mg/m², respectively. Median age was 57 years (range 20-84 years) by cytogenetics, 1, 6, and 12 patients had good-risk, intermediate-risk, and poor-risk disease. Seven patients have received ≥6 cycles; 4 remain on treatment. Two DLTs, multi-organ failure and reversible ALT elevation, were reported at the 78 mg/m² dose level. Accrual has been completed and the MTD has been established as 59 mg/m². The most common treatment-emergent grade ≥2 AEs were febrile neutropenia (n=9, 31%), elevated ALT/AST (n=5, 17%), thrombocytopenia (n=4, 14%), and pneumonia (n=3, 10%). Four patients achieved a CR. A 29-year-old woman with relapsed AML following allogeneic SCT achieved CR after Cycle 1 (25 mg/m²) before developing PD during Cycle 8. An 82-year-old man with high-risk azacitidine-refractory MDS that evolved into AML had a CR in Cycle 8 and a CRi in Cycle 10 (53 mg/m²), becoming transfusion-independent before progressing after Cycle 12. A 71-year-old man with de-novo AML refractory to standard cytaraebine plus daunorubicin induction achieved CRi during Cycle 1 (44 mg/m²); the patient has reduced transfusion independence in Cycle 15. A 51-year-old man with refractory AML following allogeneic SCT achieved a marrow CR after Cycle 1 (59 mg/m²). Plasma NEDD8 levels of MLN4924 increased linearly with increasing dose from 25-44 mg/m² after Cycle 1 Day 1 dosing in 9 evaluable patients. MLN4924 exerted predicted pharmacodynamic effects in peripheral blood and bone marrow, including increased transcription of the targets of CRL substrates in blood and the formation of MLN4924-NEDD8 adduct in bone marrow. Conclusions. NAE inhibition by MLN4924 demonstrates evidence of anti-tumor activity in patients with AML. MLN4924 appears generally well tolerated. Pharmacodynamic analyses show anticipated effects of tumor target inhibition. Further dosing schedules are being investigated to enable higher doses and greater MLN4924 exposure.

**0069**

**IDENTIFICATION OF A SUBSET OF AML CASES WITH CYTOPLASMIC NPM1 LOCALIZATION WITHOUT DETECTABLE KNOWN NPM1 MUTATIONS**

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Background. Mutation of the nucleophosmin gene (NPM1) is detected in about 30% of all patients with acute myeloid leukemia (AML). AML with mutated NPM1 shows distinctive biological and clinical features and is a provisional entity in the 2008 WHO classification. One of the important clinical features of NPM1-mutated AML is its favorable prognosis. NPM1 mutation analysis was performed by cDNA fragment analysis with a fluorescent labeled forward primer. For sequencing of the NPM1 gene RT-PCR was performed and PCR products were sequenced. Results. A total of 119 patients diagnosed with AML in the University Medical Center Groningen from 2005 to 2010, excluding those with favorable cytogenetic abnormalities, were analyzed in this study. The mean age was 57 years (range 17 to 81 yr) and 52% was female. Cytogenetics revealed that 65 patients (55%) had a normal karyotype and 26 (22%) had an unfavorable karyotype. An internal tandem duplication of the FLT3 gene (FLT3-ITD) was found in 23% of cases. Screening for NPM1 mutations by fragment analysis revealed mutated NPM1 in 34 out of 119 patients (29%). Screening for NPM1 mutations by IHC revealed cytoplasmic NPM in 33 out of 119 patients (28%) of these cases (Table 1). However, 5 cases had mutant NPM1 by fragment analysis but nuclear localization of NPM1 by IHC, and, reversely, 4 cases had no NPM1 mutation detected by fragment analysis but cytoplasmic localization of NPM1 by IHC. Additional sequencing of exons 9 and 11 of the NPM1 gene of the latter cases did not reveal mutations at these sites. Consequently, in this study considering fragment mutation analysis as a gold standard, the positive predictive value of cytoplasmic NPM1 was 89% and the negative predictive value was 94%. The sensitivity of the IHC analysis was 85% with a specificity of 95%. In addition, we performed qualitative RT-PCR studies of several HOX genes, MEIS1, PBX3, BAALC and MLL1. The cases with nuclear staining of NPM1 by IHC but a NPM1 mutation by fragment analysis showed a gene-expression profile as expected for NPM1 mutated AML. Interestingly, also the gene-expression patterns of the AML cases with cytoplasmic NPM1 by IHC but no known NPM1 mutation closely resembled those of mutated NPM1 controls. Conclusion. NPM1 mutation analysis by RT-PCR studies of several HOX genes, MEIS1, PBX3, BAALC and MLL1. The cases with nuclear staining of NPM1 by IHC but a NPM1 mutation by fragment analysis showed a gene-expression profile as expected for NPM1 mutated AML. Interestingly, also the gene-expression patterns of the AML cases with cytoplasmic NPM1 by IHC but no known NPM1 mutation closely resembled those of mutated NPM1 controls. Conclusion. NPM1 mutation analysis by RT-PCR studies of several HOX genes, MEIS1, PBX3, BAALC and MLL1. The cases with nuclear staining of NPM1 by IHC but a NPM1 mutation by fragment analysis showed a gene-expression profile as expected for NPM1 mutated AML. Interestingly, also the gene-expression patterns of the AML cases with cytoplasmic NPM1 by IHC but no known NPM1 mutation closely resembled those of mutated NPM1 controls. Conclusion. NPM1 mutation analysis by RT-PCR studies of several HOX genes, MEIS1, PBX3, BAALC and MLL1. The cases with nuclear staining of NPM1 by IHC but a NPM1 mutation by fragment analysis showed a gene-expression profile as expected for NPM1 mutated AML. Interestingly, also the gene-expression patterns of the AML cases with cytoplasmic NPM1 by IHC but no known NPM1 mutation closely resembled those of mutated NPM1 controls. Conclusion. NPM1 mutation analysis by RT-PCR studies of several HOX genes, MEIS1, PBX3, BAALC and MLL1. The cases with nuclear staining of NPM1 by IHC but a NPM1 mutation by fragment analysis showed a gene-expression profile as expected for NPM1 mutated AML. Interestingly, also the gene-expression patterns of the AML cases with cytoplasmic NPM1 by IHC but no known NPM1 mutation closely resembled those of mutated NPM1 controls. Conclusion.
Basic science in bleeding disorders

**0070**

MODULATION OF IMMUNE RESPONSES TO SELF AND NON-SELF ANTIGEN BY ACTIVATED PLATELETS IN VITRO

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**Background.** Platelets are abundantly present in the circulation, and activated platelets may have a role in regulating immune responses. Recent studies have indicated that activated platelets may stimulate maturation of monocyte-derived dendritic cells and regulate B-cell activity via shedding of soluble and platelet-derived microparticle bound CD40L. The impact of platelets on T-cell function is largely unknown, however.

**Aims.** In this study we examined how activated platelets influence the cytokine production and CD4+ T-cell proliferation elicited by self- and non-self antigens in cultures of mononuclear cells (MNCs) from healthy donors. **Methods.** MNCs were isolated from 10 healthy donors (5 females, mean age 38 years, SD ± 16 years) and labelled with carboxyfluorescein succinimidyl ester (CFSE). Platelets were isolated by centrifugation and activated by thrombin-receptor activating peptide (TRAP). The MNCs were cultured in media containing autologous serum and stimulated with tetanus toxoid (TT), thyreoglobulin (Tg), autologous platelets (plts) or combinations of TT + plts or Tg + plts. Platelets were added to the cultures in concentrations ranging from 0.5 - 3.4 x1010/L. Culture supernatants were harvested at day 1 and analysed for Th1/Th2 cytokine production. CD4+ T cell proliferation was measured at day 7 by flow cytometry. MNCs and platelets from 10 healthy donors (5 females, mean age 28 years, SD ± 10 years) were subsequently isolated and stimulated correspondingly, but were harvested after 16 hours for analysis of IL-10 secretion. **Results.** Addition of activated platelets caused an increased production of IL-10 in MNC cultures stimulated with Tg (p=0.0005), and reduced the production of TNF-a (p=0.0257). In cultures stimulated with TT, addition of activated platelets resulted in an increased production of IL-6 (p=0.012). IL-10 secretion assays revealed a significant higher percentage of IL-10 producing CD4+ T cells in cultures stimulated with Tg in presence of platelets compared to cultures containing platelets alone (p=0.045)(Fig. 1A, horizontal bars represent means). Moreover, activated platelets inhibited both the Tg- and the TT-induced proliferation of CD4+ cells (p=0.002 and p=0.037, respectively) (Fig. 1B, horizontal bars represent means). **Conclusion.** Addition of activated autologous platelets to MNC cultures increases the IL-10 production by i.a. CD4+ T cells and, accordingly, inhibits the proliferation of CD4+ T cells elicited by the self-antigen Tg in vitro. Inhibition of the CD4+ T-cell proliferation elicited by the foreign antigen TT was also observed. The results of this study suggest that platelets may have a role in regulating the adaptive immune system.

**Figure 1.** (A) IL-10 secretion (B) CD4+ T-cell proliferation.
POLYMORPHISMS IN IMMUNE RESPONSE GENES IN SEVERE HAEMOPHILIA A PATIENTS WITH INHIBITORS IN INDIA

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Background. The development of alloantibodies or ‘FVIII inhibitors’ to infused FVIII, is perhaps the most serious complication of FVIII replacement therapy. It leads to a considerable increase in mortality and cost of management of bleeds among these patients. In developing countries such as India, where resources are limited, an increased incidence of post-operative inhibitor development has been reported, which usually proves disastrous during this critical time of wound healing. A number of genetic and non-genetic risk factors have been implicated, but there is no data on the predisposing risk factors for inhibitor development in Indian haemophilia A patients. Aims. We studied polymorphisms in the II.β, IL4, IL10, CTLA-4 and TNFA genes, in 100 Indian severe hemophila A patients, i.e. 40 inhibitor positive patients (and also 20 inhibitor concordant or discordant haemophilic family members), and 40 inhibitor negative control patients, to attempt to find a marker for the differential immune response to FVIII. The polymorphisms selected for study have been shown to influence the production and function of various immune disorders, and some are reported to be associated with FVIII inhibitors in other populations. Methods. The II.β rs1145634 C/T and II.α rs2243520 C/T single nucleotide polymorphisms (SNPs); as well as the CTLA-4 rs7542909 C/T and rs281775 A/G SNPs, were analysed by the PCR-RFLP technique. Six TNFA SNPs, rs1800629 G/A, rs665252 A/G, rs1800630 C/T, rs1801282 C/A, rs1801393 A/G, rs1799724 C/T, and rs5030662 G/A were also studied in these patients by DNA sequencing. Cost-effective and quicker allele-specific PRCs have been designed to analyze the TNFA rs1799724 C/T and rs5030662 G/A SNPs. Genotyping for the IL-10 promoter dinucleotide microsatellite, IL10C, was carried out using a 6-FAM fluorescent labeled forward primer and GeneMapper analysis software (v.4.0). Results. The ‘C/T’ genotype of the TNFA rs1799724 C/T polymorphism was found to be significantly higher in inhibitor positive patients (OR: 4.645, P=0.0228, 95% CI:1.00-17.982; Fisher’s exact test). The other cytokine related gene polymorphisms showed no strong association, in contrast to reports on the association of certain polymorphisms with inhibitors in other populations. Summary/Conclusions. This is the first report from India on the association of polymorphisms in immune response genes with inhibitors in severe haemophilia A patients. These findings could influence the study of other immune response gene polymorphisms as well as other genetic risk factors of inhibitor development. This could provide useful insights into the immune response to FVIII in inhibitor positive haemophilia A patients, and possibly influence the timely prediction and prevention or treatment of FVIII antibodies.

0073 A RARE CASE OF COMBINED INHERITED DYSFIBRINOGENEMIA AND FACTOR VII DEFICIENCY FROM MUTATIONS IN THE FGB AND F7 GENES

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Background. Dysfibrinogenemia and factor (FVII) deficiency are autosomal inherited rare coagulation disorders. The genetic backgrounds of the disorders are mutations in the fibrinogen Aα (FGA), Bβ (FGB) or γ (FGG) gene and F7 gene, respectively. Clinical manifestations of both disorders are variable from asymptomatic form to life-threatening hemorrhage. Aims. We herein report the first genetically confirmed case of dysfibrinogenemia combined with FVII deficiency. Methods. The patient was a 51-year-old man referred for prolonged PT that was accidentally detected on preoperative screening. Past medical history revealed no history of bleeding tendency even on several surgeries to treat hemorrhoid, injured intervertebral disks of the lumbar spine, and sprain of right rotator cuff. Routine coagulation studies revealed prolonged prothrombin time (PT) at INR 1.1-1.5; marginally prolonged activated partial thromboplastin time (aPTT) at 14.1 sec (reference range, 29.1-41.9 sec), prolonged thrombin time (TT) at 42.2 sec (14.3-16.5 sec), and decreased fibrinogen level at 57 mg/dl (182-380 mg/dl, Claus method). Special coagulation tests revealed a decreased FVII activity of 44% (66-149%). On suspicion of dysfibrinogenemia and hereditary factor VII deficiency, direct sequencing analyses were performed for FGA, FGB, FGG and F7 genes. Results. The patient was found to be heterozygous for a point mutation in exon 8 of FGB, replacing the last stop codon to tryptophan (c.1475A>G, p.X492TrpX15). The mutation was previously reported in dysfibrinogenemia (gamma- 1547delC, Magdeburg IIB). In addition, the patient was heterozygous for a known missense mutation in exon 6 of F7 (c.466C>A, p.Gly156Ser). Collectively, the patient was confirmed to have dysfibrinogenemia and heterozygous FVII deficiency by molecular genetic studies. Summary/Conclusions. To our knowledge, the present patient is the first genetically confirmed case of dysfibrinogenemia and Factor VII deficiency. It was suggested that these autosomally inherited coagulation disorders might not be very rare and have potentially been under-diagnosed in Koreans.
This prompted us to review all available data. Methods. Systematic review of PubMed indexed literature has been performed, leading to the identification of 51 studies. 7 studies on animals and 2 on continuous infusion were not integrated, yielding to 42 analysed studies. Among these studies only 15 (56%) were in accordance with international sampling recommendations and 19 (45%) were conformed to recommendations of doses for injection. Concerning recovery (IU/dl/IU/kg), only 20/42 were considered as methodologically acceptable with data available for 26 studies. Results. Recovery data indicate for plasma-derived FIX (pdFIX) 1.17 ± 28 vs rFIX 0.81 ± 0.14 showing a significant advantage for pdFIX (p<0.0001). Only 4 paired studies were available, showing also a very clear difference between 1.41 ± 0.58 for pdFIX and 0.82 ± 0.05 for rFIX (p=0.03). The comparison of clearance (ml/kg/h) could be achieved among 22 studies for pdFIX and only 3 studies for rFIX, showing also a significant difference: 5.6 ± 1.9 for pdFIX and 8.5 ± 4.5 for rFIX with p<0.05. Conclusion. These data may explain why lower quantities may be enough for treating hemophilia B patients when using pdFIX as previously suggested.

0076 MARKERS OF COAGULATION ACTIVATION AND ENHANCED FIBRINOLYSIS IN GAUCHER TYPE 1 PATIENTS: EFFECTS OF ENZYME REPLACEMENT THERAPY

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Background. Low-grade coagulation activation and enhanced fibrinolysis have been reported in Gaucher patients (GP). Data concerning the effects of enzyme replacement therapy (ERT) on that aspect of Gaucher disease are rare and controversial. Aim. To assess the parameters of coagulation and fibrinolysis activation in our GP and the impact of ERT on them. Methods. From 2005 to 2008, 21 Serbian treatment-naïve type 1 GP (M/F 11/10, median age 37 years, range 2-74; splenectomized 8/21) were studied. Chitotriosidase activity (CA) was measured by spectrofluorometry method. Spleen volume (SV) was assessed with CT. Fibrinogen was measured according to standard methods. Plasminogen, protein C (PC), antithrombin (AT) and plasminogen activator inhibitor (PAI) were measured using spectrophotometric assay. Thrombin-antithrombin complexes (TAT), prothrombin fragments (F1+2) and D-dimer were measured by ELISA. All GP were treated with ERT (imiglucerase). Haemostatic parameters were assessed after 6, 12 and 24 months (ERT6, 12, 24). Results. Pre-treatment: Mean values of markers of coagulation activation (TAT and F1+2) and mean value of marker of enhanced fibrinolysis (D-dimer) were elevated although disseminated intravascular coagulation (DIC) was not registered (DIC score: mean 2, range 0-4). Table 1. A significant positive correlation between CA and D-dimer (p=0.047) as well as a significant inverse correlation between CA and PC level (p=0.040) were registered. Non-splenectomized GP had significantly reduced AT and PC level compared to the splenectomized ones (p=0.0285 and p=0.0599). After ERT: CA and SV significantly decreased during ERT (p=0.0001). Fibrinogen (p=0.001), TAT (p=0.0001), F1+2 (p=0.0001) and D-dimer (p=0.05) significantly decreased while PC (p=0.006) and PAI (p=0.02) significantly increased at ERT24 and remained unchanged at ERT24. At ERT24 the mean D-dimer was still elevated while the mean TAT and F1+2 remained in normal ranges. Conclusion. Our results suggest that a significant number of our GP had ongoing low-grade coagulation activation and enhanced fibrinolysis without an overt DIC, which might contribute to the bleeding tendency of GP. D-dimer and PC levels correlated with total CA and SV suggesting a relationship between Gaucher cells burden and deriving proinflammatory cytokines on one side and coagulation activation on the other side. ERT significantly decreased the level of coagulation activation and enhanced fibrinolysis.

Table 1. Markers of coagulation activation and fibrinolysis.

0077 RARE COAGULATION DISORDERS: A STUDY OF 70 CASES IN THE EGYPTIAN POPULATION

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Background. Few Rare Bleeding Disorders registries (RBD) exist, none in Africa. Rare coagulation defects, such as factor (F)II, FV, FV +FVIII, FVII, FX, FXI and FXIII deficiencies are transmitted as autosomal recessive traits with more prevalence in Muslim countries where consanguineous marriages are frequent. However, epidemiological information on the real distribution of these deficiencies is still limited and consequently when compared with the common bleeding disorders, most of these rare disorders are not well characterized clinically and do not have well-established treatment strategies. The clinical data of some rare coagulation disorders registries such as the Italian, Iranian and North American have been reported. Only several small scale studies have been reported in Africa with no data to date in the Egyptian population. Aim. To compare the clinical spectrum of some RBD in Egypt with other published data and to see if there are differences that may help in the development of many historical ethnic variations. Methods. A local hospital registry was started and patients were studied over 3 years. Assessment included detailed bleeding history, diagnostic events, clinical manifestations and treatment received. FX and FV deficiencies were classified according to the North American Rare Bleeding Disorders Registry and FVII deficiency according to the severity scoring system. Treatment was provided according to UKHCDO guidelines in view of resource constraint and patient’s previous bleeding history. Results. We report the full clinical data of 70 patients, 61.4 % males and 38.6% females with the vast majority the offspring of consanguineous marriages. Methods. A local hospital registry was started and patients were studied over 3 years. Assessment included detailed bleeding history, diagnostic events, clinical manifestations and treatment received. FX and FV deficiencies were classified according to the North American Rare Bleeding Disorders Registry and FVII deficiency according to the severity scoring system. Treatment was provided according to UKHCDO guidelines in view of resource constraint and patient’s previous bleeding history. Results. We report the full clinical data of 70 patients, 61.4 % males and 38.6% females with the vast majority the offspring of consanguineous marriages. The clinical data of some big rare coagulation registries such as the Italian, Iranian and North American have been reported. Only several small scale studies have been reported in Africa with no data to date in the Egyptian population. Aim. To compare the clinical spectrum of some RBD in Egypt with other published data and to see if there are differences that may help in the development of many historical ethnic variations. Methods. A local hospital registry was started and patients were studied over 3 years. Assessment included detailed bleeding history, diagnostic events, clinical manifestations and treatment received. FX and FV deficiencies were classified according to the North American Rare Bleeding Disorders Registry and FVII deficiency according to the severity scoring system. Treatment was provided according to UKHCDO guidelines in view of resource constraint and patient’s previous bleeding history. Results. We report the full clinical data of 70 patients, 61.4 % males and 38.6% females with the vast majority the offspring of consanguineous marriages. Methods. A local hospital registry was started and patients were studied over 3 years. Assessment included detailed bleeding history, diagnostic events, clinical manifestations and treatment received. FX and FV deficiencies were classified according to the North American Rare Bleeding Disorders Registry and FVII deficiency according to the severity scoring system. Treatment was provided according to UKHCDO guidelines in view of resource constraint and patient’s previous bleeding history. Results. We report the full clinical data of 70 patients, 61.4 % males and 38.6% females with the vast majority the offspring of consanguineous marriages. Methods. A local hospital registry was started and patients were studied over 3 years. Assessment included detailed bleeding history, diagnostic events, clinical manifestations and treatment received. FX and FV deficiencies were classified according to the North American Rare Bleeding Disorders Registry and FVII deficiency according to the severity scoring system. Treatment was provided according to UKHCDO guidelines in view of resource constraint and patient’s previous bleeding history. Results. We report the full clinical data of 70 patients, 61.4 % males and 38.6% females with the vast majority the offspring of consanguineous marriages.

0078 CARDIAC RESYNCHRONIZATION THERAPY DEVICE IMPLANTATION OR REPLACEMENT IN PATIENTS ON ORAL ANTI-OAGULATION TREATMENT. EFFICACY AND SAFETY OF A REDUCED-DOSE PROGRAM

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Background. Many patients who require cardiac resynchronization therapy (CRT) are on oral anticoagulant therapy (OAT). Current guidelines recommend discontinuation of OAT and the initiation of anticoagulant ‘bridging’ therapy with heparin during these procedures. But some patients may be exposed to bleeding and/or thromboembolic complications. Our purpose was to evaluate the efficacy and the safety of CRT-device (CRT-D) implantation or replacement without interruption of OAT, applying a dose reduction program. Methods. A group of patients attending the Cardiology Department with an indication of CRT that were previously on chronic oral anticoagulation, was prospectively...
analyzed. We collected their main baseline characteristics: age, sex, previous clinical history, blood cell counts, biochemistry (including renal function parameters), OAT indication, and concomitant use of antplatelet drug. The type (implantation or replacement) and duration of the procedure, and the type of CRT-device, were also recorded. We established the hemorrhagic-thrombotic risk of each patient. We applied a dose adjustment of the OAT to achieve, just the day of surgery, an internal normal ratio (INR) between 1.5 and 2.0 for patients at low risk of thrombosis, and about 2.0 in case of high risk. Among others, the incidence of bleeding and thrombotic complications was analyzed. For statistical analysis, we used the Fischer’s exact test for qualitative variables and the Mann-Whitney test for quantitative variables.

Results. A total of 31 consecutive patients (mean age 73 years, range 36-90, males 24) were enrolled: 14 cases of CRT-D replacements (13 pacemakers and 1 defibrillator) and 17 CRT-D implantations (13 pacemakers, 3 re-synchronizers and one defibrillator). The mean time spent in the procedure was 55 minutes (range 15-150). In 18 patients (58%) the indication for OAT was a permanent or paroxysmal atrial fibrillation, and in six (19%) a mechanical valve prosthesis. A high bleeding risk was established in 19% of patients and a medium-high thrombotic risk in 52%. Five patients (16%) were carriers of coronary stent and six patients (19%) were also taking antplatelet agents. The mean INR value on the day of surgery was 1.76 ± 0.54. Peri-procedure, 5 patients (16%) had persistent antithrombin deficiency: four cases of mild-decreased heparin activity and one mild-thrombotic complication. Forty-five days after the procedure, all complications had resolved. The baseline conditions of the patients associated with a higher rate of complications were a terminal renal failure status (p=0.037) and a prosthetic mechanical valve (p = 0.06). Results of multivariate logistic regression analysis showed that these parameters were independent predictors of complications. Significant higher rates of complications were those with advanced renal failure or mechanical valve prostheses. Five patients (16%) were carriers of coronary stent and six (19%) a mechanical valve prosthesis. A high bleeding risk in 52%. Five patients (16%) were carriers of coronary stent and six patients (19%) were also taking antplatelet agents. The mean INR value on the day of surgery was 1.76 ± 0.54. Peri-procedure, 5 patients (16%) had persistent antithrombin deficiency: four cases of mild-decreased heparin activity and one mild-thrombotic complication. Forty-five days after the procedure, all complications had resolved. The baseline conditions of the patients associated with a higher rate of complications were a terminal renal failure status (p=0.037) and a prosthetic mechanical valve (p = 0.06). Results of multivariate logistic regression analysis showed that these parameters were independent predictors of complications. Significant higher rates of complications were those with advanced renal failure or mechanical valve prostheses.

Further studies involving a larger number of cases are needed to confirm these preliminary results.
it consumes mAb and reduces therapeutic efficacy. Here, we report that the inhibitory Fc gamma receptor (FcγRIIB) on target B-cells is a key regulator of this process. The aim of the study is to uncover the mechanism behind the internalisation of rituximab. Methods. Internalisation of anti-CD20 mAb in primary tumour material was analysed using an in vitro flow cytometry-based fluorescence quenching assay. Western blotting and confocal microscopy were used to elucidate the events post-internalisation. Results. Rapid internalisation was particularly evident in most cases of chronic lymphocytic leukaemia and mantle cell lymphoma (MCL), but not from the majority of follicular or diffuse large B-cell lymphoma samples, possibly explaining their differing clinical responses to rituximab. Within each disease, the speed and extent of internalisation was heterogeneous. Internalisation of rituximab correlated strongly with FcγRIIB expression on tumour cells regardless of lymphoma subtype. Transfection of FcγRIIB converted FcγRIIB- Ramos cells into rapid internalisers in a dose-dependent manner. Internalisation also resulted in reduced macrophage phagocytosis of mAb-coated targets and could be inhibited by blocking FcγRIIB. Internalisation was largely cell-intrinsic, independent of contact with other FcγRIIB-expressing cells, in a process wherein FcγRIIB was phosphorylated and internalised along with CD20 and mAb prior to lysosomal degradation. This data is supported by clinical results showing that high FcγRIIB expression predicted less durable responses following rituximab-containing regimens in a small cohort of MCL patients. Summary/conclusions. High FcγRIIB expression provides a potential biomarker of response to rituximab-containing therapy and may identify patients for which treatment with type II anti-CD20 mAb may be preferable.

Figure 1. PFS of MCL patients by FcgRIIb expression.

0082
MONOALLELIC EXPRESSION OF THE CANDIDATE TUMOR SUPPRESSOR GENE C13ORF1 IDENTIFIED BY PROMOTER-RESTRICTED H3K4ME2
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Monoallelic expression is a mechanism of gene regulation especially relevant in the context of tumor suppressor genes (TSGs), where inactivation of the single expressed copy can lead to a complete loss of tumor suppressive function. Monoallelic gene expression has been proposed to correlate with promoter-restricted enrichment of dimethylated lysine 4 of histone H3 (H3K4me2). We used this epigenetic mark to predict monoallelic expression of candidate tumor suppressor genes in a critical region in chromosomal band 13q14.3 telomeric to RB1 that is recurrently deleted in tumors. While the biallelically expressed gene KPNAS and the monoallelically expressed candidate tumor suppressor gene RFP2 localized in 13q14.3 showed no promoter-restricted enrichment of H3K4me2, the monoallelically expressed candidate tumor suppressor mRNA genes DLEU1 and DLEU2 carry significantly more H3K4me2 marks in their promoters compared to their first exons. Interestingly, based on the promoter-restricted enrichment of H3K4me2 in peripheral blood mononuclear cells, we identified the potential tumor suppressor gene C13ORF1 to be also monoallelically expressed in a parent-of-origin independent manner. We suggest that this gene might be involved in the pathomechanism of chronic lymphocytic leukemia (CLL), where more than 50% of patients display loss of one allele in the leukemic cells and where monoallelic expression will at least in a subset of patients cause a complete loss of C13ORF1 function.

0083
TARGETED RE-SEQUENCING TO ESTABLISH FREQUENCIES OF MUTATIONS IDENTIFIED BY WHOLE GENOME SEQUENCING IN B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA
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B-cell chronic lymphocytic leukaemia (CLL) is characterised by clinical and biological heterogeneity. Its pathogenesis remains largely unknown and it is unclear which genes are involved in disease maintenance and progression. Next generation sequencing technology has the potential to analyse the whole genome for sequence variations and is a sensitive tool to elucidate the complexity of cancer genomes. We therefore performed whole genome sequencing (WGS) on 2 patients with relapsed/refractory CLL and identified multiple protein code changing mutations. The aim of the present study was to validate our findings in a larger cohort of CLL patients. Methods. Discovery WGS was performed on 20 patients and extended to an average of >30-fold coverage of blood samples of 2 patients plus matched germine buccal swab controls. Sequencing employed SBSv5 chemistry on the HiSeq2000 instrument. Paired 100-base reads were aligned to the human reference GRCh37/hg19 and candidate single nucleotide variants (SNVs), insertions, deletions and copy number variants (CNVs) were detected in both genomes. Potential candidate mutations were selected on the basis of: (1) functional annotations, (2) predicted impact on protein function using SIFT and Polyphen analysis, and (3) high-resolution SNP array data on a large cohort of CLL patients which identified copy number alterations (CNAs) affecting genomic regions in the potential candidates affected by targeted re-sequencing. Next, 40 CLL patients with treatment naïve or relapsed/refractory CLL were selected and subjected to capillary sequencing of the coding exons of candidate mutations. Results. WGS of 2 CLL patients revealed on average 20 non-synonymous, nonsense or frame-shift mutations per patient. Of the genes selected, many are involved in cancer pathways (eg MEK1, ASXL1, FAT3, NRG3) or are important in B-cell development or regulation of the innate immune response (ADAD1, SAMHD1, BCL2L13). Besides, we identified mutations in wnt pathway members. Conclusions. WGS has the potential to efficiently detect mutations in promising candidate genes involved in CLL pathogenesis. Work is ongoing to elucidate the frequency and dynamics of these mutations in a larger cohort of patients to provide insights into understanding CLL biology and help direct future therapeutic options.

0084

This abstract has been withdrawn.

0085
IMPACT OF THE MEVALONATE PATHWAY AND HIF/PGP AXIS IN MODULATING MULTIDRUG-RESISTANCE IN UNMUTATED CLL CELLS
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Background. The mutational status of the tumor immunoglobulin heavy chain variable region (IGHV) is a very reliable prognosticator in chronic lymphocytic leukemia (CLL): patients with unmutated (UM) IGHV have a worse prognosis than patients with mutated (M) IGHV. The tumor microenvironment actively supports the survival and in vivo accumulation of CLL cells and confer a multidrug resistance (MDR) phenotype to CLL B cells. MDR is due to the over-expression of membrane transporters, like P-glycoprotein (Pgp), which actively extrudes
several anticancer drugs. The activity of Pgp is under the positive control of the transcription factor Hyponxia-Inducible-Factor-1 (HIF-1α), which is in turn activated by Ras/Rho-dependent signaling. A low mevalonate pathway activity, is known to be associated to a reduced production of isoprenylated small G-proteins, which are crucial factors for Pgp activity. Little is known on the role of metabolic and molecular pathways regulating chemoresistance in CLL cells. Aims. The aim of this study was to investigate the role of metabolic and signaling pathways involved in MDR modulation in M and UM CLL cells, in order to identify specific targets of therapeutic interventions. Methods. M and UM CLL cells negatively selected by magnetic beads isolation were cultured in standard medium in the presence or in the absence of murine stromal cells (MSC) and CLL-derived stromal cells (BMSC). The Mevalonate pathway activity was measured by cells radiolabelling with [14C]-mevalonic acid, lipid extraction and thin layer chromatography. RhoA/Ras isoprenylation, ERK1/2, Akt activity and HIF-1 phosphorylation were detected by Western blot. Pgp expression was measured by Real Time-PCR and Western Blot and its activity was evaluated by measuring the efflux of rhodamine 123. The Cytotoxicity induced by Doxo was analysed by annexin V and propidium iodide (PI) staining. Results. UM CLL cells showed a significantly more active Mev pathway than M CLL cells. The higher levels of Mev pathway activity was associated to a higher expression of Ras/Rho and to a higher activity of downstream kinases Akt and ERK1/2. This higher activity of ERK/Akt pathway was paralleled by a higher expression of phosphorylated HIF-1α, which is a potent inducer of mdr1/Pgp gene. As expected, UM CLL cells displayed higher levels of mdr1 mRNA expression, lower accumulation of intracellular Doxo and higher in vitro viabilities in Doxo exposure. BMSCs induced the upregulation of Rho/Rho Kinase and Pgp proteins in UM CLL cells, protecting them from Doxo-induced cytotoxicity. Targeting of the Mev pathway by Zoledronic acid determined a significant reduction of the Rho/Rho kinase and Pgp activity, and abrogated BMSC-related chemoresistance. Conclusions. Our data demonstrate that Hif1/Pgp axis is more active in compared to M CLL cells, leading to higher levels of chemoresistance. Specific targeting of the Mevalonate pathway and/or downstream signalling pathways may represent a promising strategy of therapeutic intervention.
type IGHV1-69 and subset #3 (IGHV1-69/IGHD2-2/IGHJ6) patients. Conclusion. Our findings suggest that distinct stereotyped BCRs in CLL are characterized by specific cytogentic profiles and clinical course. In particular, we found a significant prognostic relevance of subset #1 in the context of UM CLL patients that would be validated in larger prospective series of patients.

**0088 SNP BEAD-ARRAYS: TOWARDS A ROUTINE CLINICAL USE IN CHRONIC Lymphocytic Leukemia (C-LL)**

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Chronic lymphocytic leukemia (C-LL) cells convert CD14+ cells from healthy donors into cells with morphological similarities to NLCs (CD14CLL-cells). However it is unclear whether this conversion process is only induced by C-LL cells. We show that CD14+ PBMCs from healthy donors can also be converted into differentiated cells (CD14B-cells) by non-malignant B-cells. Intriguingly, non-malignant C-LL sorted B-cells were not able to differentiate CD14+ PBMCs from healthy donors into cells (CD14B-cells) that support survival similar to NLC and CD14CLL-cells. Moreover, gene expression levels of survival-associated genes like APRIL, BAFF and PECAM1 were comparable in all three differentiated cell types (NLC, CD14CLL- and CD14B-cells). In order to identify changes specifically induced by C-LL cells, we compared gene expression profiles of NLCs, CD14CLL-cells and CD14B-cells. CD14+ cells cultured with C-LL cells were more similar to NLCs than those cultured with non-malignant B-cells. The most significant changes induced by C-LL cells were deregulation of the antigen presentation pathway and of genes related to immunity. NLCs had reduced levels of lysosomal activity, CD74 and HLA-DR in-vitro while expression of inhibitory FcγR2B was increased. These findings suggest an impaired immunocompetence of NLCs which, if found in-vivo, could contribute to the immunodeficiency in C-LL patients.

**0089 PHOSPHORYLATION LYMPHOCYTIC LEUKEMIA CELLS THROUGH TYR705STAT3**

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**Background/Aims.** Determine if SNP random bead-arrays (Illumina, using oligonucleotides probes immobilized onto beads, that correspond to Single-Nucleotide Polymorphisms along the human genome) are more sensitive than Interphase Fluorescence In Situ Hybridization (I-FISH) or Conventional metaphase Cytogenetics (CC) to detect specific chromosomal abnormalities associated with prognosis in B-CLL, e.g. deletions at 17p, 11q, 13q and trisomy 12. Methods. A blood sample from 24 patients with B-CLL at diagnosis was analysed simultaneously by I-FISH, CC and SNP bead-array. FISH: LSI p53, ATM and CEP12 Single Color Probes (Abbott) were used, to detect del(17p13.1), del(11q22.3) and tris 12, respectively. For detection of del(13q14.3), we tested in parallel the LSI D13S319 (~130 kb) Single Color (Abbott) and 13q14.3 specific Dual Color probes (CytoCell). In normal lymphocytes, an average of 6% nuclei showed a loss of signal (truncated nuclei) or random colorized signals, and the cut-off to ascertain a chromosomal deletion was defined over 10% (n=450,000) CC: B-Lymphocytes are cultivated for 72 hours with immunosstimulants (DSF05 and IL-2) SNP DNA (200 ng) was hybridized on the Illumina Human Mapping 550 K Bead Chip (~575,977 markers; 4.9 kb median marker spacing; mean 7.8 kb). Results. FISH identified del(13q) (19-96% of nuclei, m=47%, del(11q) (55-54%) and tris12 (25-49%) in 18 (75%) [including 2 biallelic deletions], 3 (12.5%) and 4 cases (17%) respectively (5 cases presented associated abnormalities). SNP-arrays detected all of those abnormalities induced by the osteoblast-like cell line MG63 in co-culture experiments. We further observed that co-culture of CLL cells with conditioned media from BMSC or MG63, or their treatment with recombinant HGF produced a significant inhibition of the prolonged survival of CLL cells by the use of an anti-HGF neutralizing antibody or of the c-MET inhibitor SU11274 in experiments where CLL cells were co-cultured with different mesenchymal cell lineages. Silencing of HGF production by siRNA in mesenchymal cells. Moreover through western blot and cyto-fluorographic analysis we studied the activation of signaling molecules potentially induced by CLL/MSC co-cultures or HGF treatment of leukemic B cells. We demonstrated a significant inhibition of the prolonged survival of CLL cells by the use of an anti-HGF neutralizing antibody or of the c-MET inhibitor SU11274 in experiments where CLL cells were co-cultured with different mesenchymal cell lineages. Silencing of HGF production by siRNA significantly reduced the anti-apoptotic effect induced by the osteoblast-like cell line MG63 in co-culture experiments. We further observed that co-culture of CLL cells with conditioned media from BMSC or MG63, or their treatment with recombinant HGF induced phosphorylation of STAT3 in TYR705 after 10-40 min. A putative role played by pSTAT3 in CLL survival by MSC co-culture or HGF treatment was further confirmed by blocking CLL cells viability by a STAT3 inhibitor. Our data underline a pivotal role of HGF in prolonging CLL cells survival and potentially in contributing to apoptosis resistance of the leukemic B cell clone at the level of particular bone marrow niches. Molecules involved in the HGF/c-MET signaling pathway(s) may be further forseen as possible therapeutical targets in the treatment of chronic lymphocytic leukemia.
HETEROGENEOUS FUNCTIONAL OUTCOMES AFTER TOLL-LIKE RECEPTOR STIMULATION IN DIFFERENT SUBGROUPS OF CHRONIC LYMPHOCYTIC LEUKEMIA ARE UNDERLINED BY DISTINCT TRANSCRIPTIONAL PROGRAMS

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We have recently reported that subgroups of patients with chronic lymphocytic leukemia (CLL) defined by distinctive molecular characteristics of the clonotypic B cell receptors (BcRs) exhibit distinct expression profiles of the Toll-like receptor (TLR) signaling pathway-associated genes as well as differential outcomes after TLR stimulation. The aim of this study was to investigate whether specific TLR-induced transcriptional modulation is observed and if it may be implicated in differential functional effects. To this end we analyzed 4 CLL cases each belonging to stereotyped subset #1 (unmutated IGHV1/5/7-IGKV1(D)-39 BcRs) or #4 (mutated IGHV4-34/IGKV2-30 BcRs). We selected these particular cases prompted by our recent finding of subset-biased functional outcomes; specifically, distinct transcriptional changes are found in the case #4. In conclusion, the TLR pathway is competent for signaling in CLL with different stereotypes and viceversa. None of the normal stereotypes associated with the CLL stereotypes and vice versa. None of the normal IGHV patterns were found in 62/165 normal (37.6%) and 51/78 CLL (65.4%, p<0.001).

Identification of 'Stereotypic' Patterns Exclusive of Unmutated IGHV1-69-Derived CLL B-cells

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Background. Chronic lymphocytic leukemia (CLL) cells expressing unmutated (UM) IGHV, a phenotype associated with aggressive clinical courses, can be more efficiently induced to proliferate by stimulating Toll-like Receptor 9 (TLR9) with unmethylated CpG oligonucleotides (CpG) than mutated (M) CLL cells. MicroRNA (miRNAs) are 18- to 22-nucleotide-long non-coding RNA molecules that regulate gene expression and play a key role in several biological processes including oncogenesis. Aim: Elucidating miRNAs involvement in regulating activation/proliferation processes of CLL cells. Methods: Freshly-isolated negatively-selected CLL cells from 17 CLL patients (9 UM and 8 M) were stimulated with CpG (18 hours) or left unstimulated. miRNA and mRNA expression were evaluated by real time-PCR and performed by qRT-PCRs.

0093 The Mir-17-92 Cluster Family Determines the Responsiveness of Chronic Lymphocytic Leukemia (Cll) Cells to Toll-like Receptor 9 Triggering

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0092 Background. Chronic lymphocytic leukemia (CLL) cells expressing unmutated (UM) IGHV, a phenotype associated with aggressive clinical courses, can be more efficiently induced to proliferate by stimulating Toll-like Receptor 9 (TLR9) with unmethylated CpG oligonucleotides (CpG) than mutated (M) CLL cells. MicroRNA (miRNAs) are 18- to 22-nucleotide-long non-coding RNA molecules that regulate gene expression and play a key role in several biological processes including oncogenesis. Aim: Elucidating miRNAs involvement in regulating activation/proliferation processes of CLL cells. Methods: Freshly-isolated negatively-selected CLL cells from 17 CLL patients (9 UM and 8 M) were stimulated with CpG (18 hours) or left unstimulated. miRNA and Gene Expression Profiling (GEP) were performed with Applied Biosystems TaqMan Low Density Arrays. In-silico prediction of miRNAs modulation was made analyzing GEP data by the T-REX and GSEA software; validations were performed by qRT-PCRs. Results: miRNA profiling and GEP were separately evaluated in UM and M-CLL. In UM-CLL, 21 miRNAs resulted up-regulated and 3 down-regulated and GEP underlying GEP data analysis selected 30 significant miRNAs, upregulated in CpG-stimulated cells, as well downregulated in CpG-stimulated cells, as well as being responsible for GEP perturbation, of which 3 of them (miR-17, miR-20a, and miR-20b) in common with the miRNA signature of CpG-stimulated cells. These 3 miRNAs and additional 4 miRNAs

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(miR-17\(^{\text{a}}\), miR-18a, miR-19b-1\(^{\text{a}}\) and miR-92a-1\(^{\text{a}}\)) again comprised in the miRNA signature, all belonged to the miR-17-92 cluster, a well-conserved miRNA cluster over-expressed in a variety of B-cell lymphomas. The GEPI of CpG-stimulated UM-CLL cells included genes involved in cell cycle regulation and NF-kappaB cascade, in agreement with the proliferative effect of CpG in UM-CLL cells. In particular, among these genes: i) E2F5, TP53INP1, TRIM8, and ZBTB4, all target of miR-17, were downregulated in CpG-stimulated cells and knock-down upon miR-17 transfection in six primary UM-CLL cells; ii) the miR-17 gene regulator MYC and the MYC target genes CAD, PKG1 and TFAM were upregulated in CpG-stimulated UM-CLL cells. Time-course experiments using primary UM-CLL cells showed that MYC and miR-17 were both upregulated upon CpG stimulation although follow different kinetics. In M-CLL cells no miRNA was differentially expressed between CpG-stimulated and unstimulated CILL cells. Despite this, in a hierarchical cluster driven by the UM-CLL microRNA signature, 4/8 M-CLL cases showed a microRNA profile similar to that of UM-CLL, including the increase of miR-17-92 cluster. In agreement with this data, the same 4 M-CLL clustered ‘near’ to UM-CLL cells in a hierarchical cluster made using the GEPI data of UM-CLL cells (“CpG-responder” M-CLL). Consistently, in these “CpG-responder” M-CLL, MYC gene resulted upregulated along with two (CAD and PKG1) MYC target genes. Conclusion: Altogether, our data suggest that TLR9 triggering is able to elicit a complete response upregulation of MYC, miRNAs from the miR-17-92 cluster family and specific gene targets for these miRNAs both in UM-CLL cells and a subset of M-CLL.

\[ \text{This abstract has been withdrawn.} \]

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**B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA CELLS EXPRESS FUNCTIONAL DEATH RECEPTOR 3 FOLLOWING STIMULATION OF THE B CEll RECEPTOR**

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**Background.** We previously demonstrated that the phosphorylation status of Hematopoietic cell specific Lyn substrate 1 (HS1) is a potential prognostic marker as CLL patients whose leukemic cells carry phosphorylated HS1 have a better prognosis than patients with the hyperphosphorylated form; ii) in the CLL-prone Eμ-TCL1 transgenic mouse is a pivotal molecule in the signal transduction pathway triggered by the BCR receptor (BCR), as well as a central interactor of several cytoskeletal components and being involved in tissue trafficking and homing and iii) has a profound effect on the development and progression of murine CLL suggesting that the hyper-phosphorylation of HS1 leads to its inactivation rather than activation. Aims. Given the role played by HS1 in BCR signalling and cell migration, we decided to dissect the signalling pathway and the migratory capacity of CLL cells presenting a differential phosphorylation status of HS1. Methods. We started by taking advantage of the CLL cell line MEC1 where we silenced the expression of HS1 and dissected the signalling pathway by western blot and flow cytometry. We then utilized primary CLL cells to validate the results. Finally we investigated in vivo the migration of human leukemic cells labeled with different concentrations of CFSE and injected intravenously into Rag2-/-/egr-/- recipient mice. Results. The HS1 silenced MEC1 cell line we found that the phosphorylation status of several BCR signalling molecules, including Lyn Kinase, SHP proteins, ERK Kinal, phosphatase Rac, and HIP-55, is directly affected by the absence of HS1. We confirmed a similar pattern of modifications in primary cells from 25 patients who had a different phosphorylation status of HS1. These findings indicate that the pattern of HS1 phosphorylation is associated with a specific biochemical signature characterized by the loss of phosphorylation in several BCR downstream signalling molecules. We then investigated the homing ability of CLL cells from patients showing different HS1 levels of phosphorylation. CLL cells purified from 8 patients were paired into 4 couples according to their HS1 phosphorylation status. Each couple included a case with phosphorylated HS1 and a case with hyperphosphorylated HS1 CLL cells. In 3/4 couples of differentially phosphorylated paired patients, CLL cells with phosphorylated HS1 had a consistent homing rate to the spleen while CLL cells with hyperphosphorylated HS1 had a preferential homing to the BM. This observation indicates a correlation between the status of HS1 phosphorylation and the propensity of human CLL cells to accumulate in the bone marrow in vivo. Conclusions. These findings strengthen our previous observation that HS1 phosphorylation has an important role in controlling cell migration and homing of leukemic B cells, likely through its involvement in cytoskeleton organization and BCR signalling. According to our future plans are: i) to increase the number of patients studied in order to detect potential correlations between signalling results, clinical behaviour and biological prognostic factors and ii) to identify novel intracellular HS1 partners to explore the possibility that the phosphorylation of HS1 may be modulated for therapeutic purposes.

\[ \text{0096} \]

**HS1 PHOSPHORYLATION, A PROGNOSTIC MARKER OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL), DEFINES A DISTINCT SIGNALING PATHWAY AND INFLUENCES THE CELL MIGRATORY CAPACITY**

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**PROGRAMMED DEATH-1 IS A NOVEL IMMUNOTOLERANT MOLECULE EXPRESSED ON LEUKEMIC B CELLS IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background.** Programmed death-1 (PD-1) molecule is an immunoreceptor, which through a interaction with its ligand (PD-L1), controls peripheral tolerance by limiting activation, development and effector function of T lymphocytes. PD-1/PD-L1 pathway was found to be one of the potential tumor escape mechanism from the immunosurveillance and PD-1 expression was described on exhausted T lymphocytes. **Aim.** We assessed a mRNA level and surface expression of PD-1 and PD-L1 in CLL patients since the aberrant expression of PD-1 might represent a novel target on the lymphocytic B-cells. It might also define possible prognostic markers. **Patients and Methods.** Quantitative reverse transcriptase PCR was performed for the PD-1 transcript with four splicing variants as well as for PD-L1 and one splicing variant in 43 CLL patients and 10 healthy volunteers (HVs). For characterization of the surface expression of PD-1 and PD-L1 on leukemic B-cells in a group of 33 CLL patients and 10 HVs magnetic separation followed by five parameter flow cytometric analysis was used. **Results.** The median level of PD-1 transcripts in CLL patients was higher in comparison with HVs (p=0.006). Additionally, expression of truncated PD-1 splicing variant lacking exon 2, 3 and 4 was lower in CLL patients compared with HVs (p=0.0465). No difference of PD-L1 expression between CLL patients and HVs on mRNA level was observed. The expression of a non-functional splicing variant lacking exon 2 was elevated in CLL patients compared with HVs (p=0.008). In flow cytometric analysis, we confirmed the presence of PD-1 and PD-L1 on the CLL cells surface. Notably, both PD-1 and its ligand were expressed on the same CLL cells. Mean fluorescence intensity (MFI) was higher among CLL patients in comparison with HVs (13.34 vs 4.9, p<0.001). There was no difference in MFI levels of PD-L1 between CLL patients and HVs. Significantly higher MFI levels of PD-L1 were observed in early stage CLL patients (13.34 vs 4.9, in stage A and stage C according to Binet classification, respectively). There was no difference in time to progression and overall survival in groups of patients characterized by low and high PD-1 and PD-L1 expression. **Conclusions.** PD-1, which is expressed both on mRNA and cell surface levels in CLL cells might represent a novel immunotolerant molecule involved in the pathomechanism of disease, as well as provide a novel target for future therapies.
**0100**

**RITUXIMAB IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ADVANCED CHRONIC LYMPHOCYTIC LEUKEMIA WITH FLUDARABINE + TBI CONDITIONING: RESULTS OF A PHASE II PROSPECTIVE MULTICENTER STUDY**

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Aims and Methods. To evaluate the efficacy and toxicity of RIC regimen including fludara. and TBI with the introduction of rituximab for allo-HSCT, we conducted a prospective study for CLL patients aged < 65 years in stage B or C in response after a salvage treatment, having a HLA identical sibling donor. The conditioning included: rituximab 37.5 mg/m2 on day-5, fludarabin e 30 mg/m2 from day-4 to day-2, TBI 2gray on day0 and rituximab 500 mg/m2 on day 1 and day 8. Forty patients were included, 34 (85%) males and 6 females, median age 54 years (35-65), 38 (95%) in B stage and 2 in stage C. Among 23 explored for cytogenetics, 8 were abnormal. Before transplantation, 17 patients received 2 lines treatment, 10 three lines, 13 5 lines. At time of allograft, 7 (17%) patients were CR, 29 (73%) in PR and 4 (10%) < PR, 59% were sex-mismatched. For ABO matching, 68% were compatible, 19% major incomp. & 13% minor incomp. The median interval diagnosis-allo-HSCT was 58 months (6-177). Seven (17%) patients did not receive rituximab during conditioning because the protocol did not include it at the beginning and has been amended later. Results: Thirty-nine (98%) patients engrafted with a median time to neutrophils recovery of 20 days (11-70), 79% of patients reached a total donor chimerism at day 90. Seventeen patients developed aGVHD grade II (3 grII, 8 grIII & 1 grIV) with a cumulative incidence at 3 months of 44% (36-52). The cumulative incidence of cGVHD was, at 12 months: 29% (21-36) for lim. and ext.; at 18 months: 32% (24-40) lim. and 42% (34-50) ext. After a median follow-up of 28 months (8-71), the median OS was not reached with 5-years probability of 55% (41-74). The median time of EFS was 30 months (15 - 70) with a 5-years probability of 46% (33-66). The cumulative incidence of relapse at 1 and 3 years was 17% (11-23) and 22% (15-29) respectively. The cumulative incidence incidence TRM at 1 and 3 years was 10% (5-15) and 27% (20-35) respectively. At the last follow-up, 17 patients died, 6 due to relapse and 11 due to TRM. The multivariate analysis showed a positive impact of rituximab on OS and EFS [HR=0.1 [0-0.6] p=0.02 & HR=0.1[0-0.4] p=0.035 respectively. Conclusion. The introduction of rituximab allowed a better outcome especially a significant reduction of incidence and severity of acute GVHD. Nevertheless there was still a high incidence of cGVHD, leading us to propose either to increase the number of rituximab injections after allo-HSCT, or to test Fludarabine/busilvex/ATG associated to rituximab.

**0101**

**RESULTS FROM A PHASE II STUDY OF OBINUTUZUMAB (GA101) MONOTHERAPY IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)**

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Background. Obinutuzumab (GA101) is the first type II, glycoengineered, humanized monoclonal anti-CD20 antibody to enter clinical trials. In a phase I study (BO20999), GA101 was administered as monotherapy to 13 patients (400-2000mg) with relapsed/refractory CLL with GA101 given on Days 1, 8 and 22 and q21 (total of 9 infusions). End of treatment response (EOR) was 62% (8/13, all PR) (Cartron, EHA 2009, Morschhauser, ASH 2009). Methods. In the phase II part of BO20999, twenty patients with relapsed/refractory CLL received 1000mg GA101 monotherapy administered (flat dose) on Days 1, 8, 15 and 22 and q21 (total of 10 infusions). Primary endpoint was EOR with responses based on combined EASL-can and hematology results (IWCLL criteria, 2008). Results. GA101 was well tolerated with the most common AE being infusion related reactions (19/20 patients, grade 1,2: n=13, grade 3 n=5, grade 4 n=1), mainly during the first infusion. Thirteen patients completed all scheduled cycles. Reasons for 7 early withdrawals were: AEs [5], insufficient response [2], patient choice [1], protocol violation [1]. Most common related grade 3/4 hematological toxicities were neutropenia (n=4) and thrombocytopenia (n=2). There was one patient who developed febrile neutropenia (resolved without sequelae). Baseline characteristics and response are shown in Table 1.

**Table 1. BO20999 - Phase I & II CLL baseline demog.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Phase I</th>
<th>Phase II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65 ± 10</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>12:9</td>
<td>14:6</td>
</tr>
<tr>
<td>ECOG (0-3)</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Prior lines</td>
<td>3 (1-5)</td>
<td>3 (1-5)</td>
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<tr>
<td>Prior ritux</td>
<td>56%</td>
<td>50%</td>
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<tr>
<td>Prior chemo</td>
<td>46%</td>
<td>40%</td>
</tr>
<tr>
<td>Prior bio</td>
<td>30%</td>
<td>20%</td>
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<tr>
<td>Prior donor</td>
<td>23%</td>
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**Figure 1.** Compared to the patient population in the previously reported phase I part of the study, patients had a higher tumor load with a median sum of product of diameter (SPD) of 2124 mm² (1,065-26,732 mm²) for the phase I patients, compared with 3138 mm² (330-6,399 mm²) for the phase II patients. Based on 16 patients evaluable by combined CT-scan and hematology results, EOR was 25% (4 PR, 5 SD, 7 PD). Importantly, peripheral B-cell depletion for all patients was rapid following the first infusion of GA101, with all patients achieving B-cell levels below 40/10 within one week of treatment, levels which were sustained during the treatment period, confirming the same observation reported in the phase I study. Of note, baseline tumour burden (TB) may be an important co-variate impacting patient response in the monotherapy setting in heavily pre-treated patients. Of the 4 PR patients, three had...
baseline TB ranging from 1383-1899 mm². Of the 6 patients with disease progression and 6 patients with stable disease, baseline TB ranged from 3004-5948 mm² and 2410-6266 mm² respectively, suggesting that those patients with baseline TB approximately >2400 mm², may not respond to the antibody when given as monotherapy, although all patients achieved impressive and sustained peripheral B-cell depletion. This is also supported in the phase I results, with 6 of the 8 responding patients having baseline TB ranging from 1068-2029 mm². This important data supports combination with chemotherapy in patients to maximize the clinical potential of GA101 in CLL to overcome lymphadenopathy. Conclusion. These phase II results indicate that GA101 has promising single agent activity in a heavily pre-treated patient population and will continue to be evaluated as a single-agent and in addition, the 1000mg dose is currently being investigated in combination with chemotherapy in a first line, phase III CLL trial (CLL-11).

LOW DOSE ALEMUTUZUMAB IS SAFE AND EFFECTIVE IN FLUDARABINE-REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Refractoriness to fludarabine-based chemo-immunotherapy is associated to poor prognosis in chronic lymphocytic leukemia (CLL). Alemutuzumab has been shown to be effective in this category of patients although associated with relevant toxicity in terms of infections. Aim. We evaluated efficacy and safety of alemutuzumab subcutaneously administered at lower dose in a cohort of fludarabine-refractory CLL patients. Methods. Thirty-nine fludarabine-refractory patients have been enrolled at our center and treated with subcutaneous alemutuzumab. Patients received 10 mg of alemutuzumab three times weekly for 18 weeks. In 18 randomly selected patients, after obtaining lymphocyte count reduction of 1 Log, the antibody was then administered once weekly at the dose of 80 mg. Biological prognostic markers were tested in 33 patients, including CD38 and ZAP70 expression by flow-cytometry, FISH panel for del(17p)(18%), del(11q)(22%), trisomy 12 and del(13q)(14); IGHV mutational status was available in 10 patients. Results. Median age was 64 years (range 48-82 years) with 31% of the patients older than 70 years. The patient population was characterized by high-risk biologic disease profile as shown by the incidence of del(17p) (18%), del(11q) (22%), unmaturated IGHV (80%), CD38+ (40%) and ZAP70+ (55%). Median previous therapy lines were 3 (1-6); twenty-one were pre-treated with FCR(8). Low-dose alemutuzumab yielded a 44% (95% CI 23.0-64.2%) overall response rate (ORR) whereas complete remission (CR) was obtained in 3 patients (8%; 95% CI 0.1-21.1%). Two of the three patients in CR resulted MRD negative. Median overall survival (OS) and progression free survival (PFS) were 29.1 months (95% CI 21.7-39.0) and 10.3 months (95% CI 8.3-16.2), respectively. Both PFS (p<0.0001) and OS (p=0.04) were significantly longer in responding versus non-responding patients. Treatment was well tolerated: all and 3-4 grade non-CMV infection were 46% and 7%, respectively. CMV reactivation occurred in 27% of the patients, showing only one case of disease. Moreover, no death occurred during therapy. No significant difference both in terms of safety and efficacy was observed in elderly (>70 years) patients and between the two different schedules evaluated. Conclusion. Our data indicate that low dose alemutuzumab is safe and effective in this setting of poor prognosis CLL patients. Efficacy results appear to be similar to those obtained with standard treatment, while toxicity is significantly lower. This data question the use of standard dose alemutuzumab in frail or elderly patients and provide evidence for an equally effective and more practical one-weekly alemutuzumab schedule.
Figure 1. CLL10 Screening Failures.
tions. We have previously reported that alemtuzumab given at lower doses may be equally effective and less toxic in refractory CLL. Aim: We conducted a multicenter retrospective study on the routine clinical use of low-dose alemtuzumab in relapsed/refractory CLL. Methods: One hundred and eight patients from 11 Italian centers were included in the analysis. Low-dose alemtuzumab was defined as a total weekly dose <45 mg and a cumulative dose <600 mg up to eighteen weeks of therapy. Both subcutaneous (SC) and intravenous (IV) administrations were allowed. The other inclusion criterion was that patients had to be treated with at least one previous line not containing alemtuzumab as single agent or in combination. Biological prognostic factors including CD38 and ZAP70 expression, as well as FISH analysis were available for 76% of patients and a recent (2014) retrospective analysis showed that alemtuzumab given at lower doses is a valid and currently used therapeutic option for the treatment of relapsed/refractory CLL, in particular in elderly and frail patients. This retrospective analysis shows that alemtuzumab given at lower doses is a valid and currently used therapeutic option for the treatment of relapsed/refractory CLL. In 37 patients (34%), with only one case of CMV infection. The overall response rate was 86% (45% CR and 41% PR), was administrated a second cycle of cladribine which led to a CR. Thirteen patients achieved a clinical remission in the absence of minimal residual disease. The median follow up was 27.6 months. The median time to treatment failure (TTF) was 36.7 months. There was a statistically significant difference in terms of duration of response between untreated and pre-treated patients (TTF respectively: not reached vs 23 months, p=0.065). Patients achieving a CR had a longer response duration than patients with PR (TTF respectively: not reached vs 23 months, p=0.025). Low serum lactate dehydrogenase levels and 2-microglobulin levels at baseline and a normal CT scan at the end of therapy (independent of response) did not influence TTF. Ten patients developed severe neutropenia (grade 3-4). Thrombocytopenia grade 3 was observed in one patient and anemia grade 2 in one patient. Five pts developed grade 4 infections (neutropenia, pneumonia, septic shock). One patient developed febrile neutropenia with diarrhoea (Salmonella positivity). In 3 patients we observed cutaneous toxicity with resolution after the use of oral steroids. Conclusions. Our data confirm that combination therapy with R-cladribine is an effective and safe treatment for patients with CLL and SLL. Achieving results similar to those reported with more aggressive regimens.

0108

LONG-TERM FOLLOW-UP OF PATIENTS WITH HAIRY CELL LEUKAEMIA TREATED INITIALLY WITH PENTOSTATIN OR CLADRIBINE. SPANISH EXPERIENCE (BY THE SPANISH COOPERATIVE GROUP ON CLL)

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9 Hospital U. Amano de Vilanova, Valencia, Spain
10 Hospital de Sagunto, Sagunto, Valencia, Spain
11 Hospital Virgen del Puerco, Plasencia, Spain
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Background. Hairy cell leukaemia (HCL) is a rare indolent lymphoproliferative disorder characterised by infiltration of the bone marrow, liver and spleen by malignant B-cell with hairlike cytoplasmic projections (HC). Purine analogues are highly effective in HCL with overall responses in more than 85%. Although, HCL patients are projected to progress in their life-span or, in a minority are refractory. Aims. To investigate the long-term outcome for patients with HCL treated with purine analogues, to compare efficacy of pentostatin and cladribine, to identify factors associated with response, or relapse. Methods. We reviewed clinical and laboratories data retrospectively from 75 patients, initially treated with pentostatin or cladribine, to investigate the current long-term outlook. Patients entering the study received pentostatin 4 mg/m2/15 days or cladribine (by different schedules) as a single course. Minimal residual disease (MRD) was studied by flow cytometric immunophenotyping in peripheral blood (PB) and bone marrow (BM). Efficacy endpoints were response to therapy, treatment free interval (TFI) and overall survival (OS). TFI was measured in all patients from treatment initiation to time of new treatment requirement. OS was measured from therapy initiation to death or last follow-up. We studied risk factors associated with response or relapse. Analysis was performed using the SPSSv15.1 software package. Results. Of 75 patients, 54 were males (72%). Median values and range were: age 54 years (31-82), haemoglobin 11,2 g/dL (4,1-15,8), platelets 73x109/L (13-267), WBC 2,7x109/L (0,7-20), %HC in BM 36 (5-90) and size spleen 15cm (12-53) without significant differences between two groups of treatments. Twenty one patients received pentostatin and 54 cladribine for first line therapy. The median follow-up was 156 months (4-393) for pentostatin group and 65 months (3-191) for cladribine group. The overall response rate was 100%. The complete remission (CR) rate was 95,2% with pentostatin and 90,7% with cladribine. The median numbers of pentostatin injections required to achieve CR were 7 (2-14). In 5 patients treated with cladribine who still remained in partial remission (PR), was administrated a second cycle of cladribine which led to a CR in 4 patients. MRD was positive in 52,4% of pentostatin patients and 33,3% of cladribine patients. Twenty patients relapsed: 47,6% patients treated with pentostatin and 18,5% of those who received cladribine. By univariate analysis risk factors of relapse were: HB (p=0,001), %HC in BM (p=0,013) and size spleen (p=0,003). Cox multivariate analysis revealed HB and splenomegaly as independent risk factors of relapse. 

0107

RITUXIMAB AND SUBCUTANEOUS CLADRIBINE: CLINICAL RESULTS OF AN EXTENDED COHORT OF CHRONIC LYMPHOCYTIC LEUKAEMIA

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Background. Rituximab in combination with purine analogue is a well established treatment for chronic lymphocytic leukaemia (CLL). Recently we published preliminary results on CLL patients using subcutaneous cladribine in combination with rituximab. Aims. Here we report updated results on 67 patients with active CLL or small lymphocytic lymphoma (SLL) treated with this combination treatment. Methods. Sixty-seven patients with active CLL or small lymphocytic lymphoma (SLL) received rituximab 375 mg/m2 on day 1 and cladribine 0.1 mg/kg subcutaneously on days 2-6. The treatment was repeated every 4 weeks for a total of four cycles. Response to treatment was evaluated according to NCI-WG updated guidelines for patients with CLL and with Cheson criteria for SLL patients. Results. Forty-five patients were previously untreated. The median age was 65 (52-78). 2-microglobulin was abnormal in 54% of patients and CD38 and ZAP70 being refractory. Different multiple genetic alterations at FISH analysis and 66% of patients showed a ZAP-70 positivity. Sixty-five patients were evaluable for response. The overall response rate was 86% (45% CR and 41% PR), with 44% of untreated patients and 41% of pre-treated patients achieving a CR. Thirty patients achieved a clinical remission in the absence of minimal residual disease. The median follow up was 27.6 months. The median time to treatment failure (TTF) was 36.7 months. There was a statistically significant difference in terms of duration of response between untreated and pre-treated patients (TTF respectively: not reached vs 23 months, p=0.065). Patients achieving a CR had a longer response duration than patients with PR (TTF respectively: not reached vs 23 months, p=0.025). Low serum lactate dehydrogenase levels and 2-microglobulin levels at baseline and a normal CT scan at the end of therapy (independent of response) did not influence TTF. Ten patients developed severe neutropenia (grade 3-4). Thrombocytopenia grade 3 was observed in one patient and anemia grade 2 in one patient. Five pts developed grade 4 infections (neutropenia, pneumonia, septic shock). One patient developed febrile neutropenia with diarrhoea (Salmonella positivity). In 3 patients we observed cutaneous toxicity with resolution after the use of oral steroids. Conclusions. Our data confirm that combination therapy with R-cladribine is an effective and safe treatment for patients with CLL and SLL. Achieving results similar to those reported with more aggressive regimens.
By Kaplan-Maier estimates the median TFI was 96 months in pentostatin, and 144 in cladribine patients; 95 months in MRD+, and 216 in MRD- patients. Conclusions. Although both agents are effective in HCL, the results are better for cladribine than pentostatin respect rate of relapse, TFI and MRD. Haemoglobin levels and splenomegaly are confirmed as independent risk factors of treatment failure and rapid progression of the disease. The relation between MRD+ and shorter TFI suggests the convenience to add treatment, like Rituximab, to obtain MRD negative response.

0109
SMALL LYMPHOCYTIC LYMPHOMA (SLL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): A SINGLE DISEASE WITH SIMILAR PROGNOSIS?
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Introduction. Small lymphocytic lymphoma (SLL) is a mature (peripheral) B-cell neoplasm characterized by a progressive accumulation of monoclonal B lymphocytes. It is considered to be identical to chronic lymphocytic leukemia (CLL) with similar pathologic and immunophenotypic features. Dissection is usually made based upon clinical presentation and an arbitrary cut-off of 5x109/L lymphocytes. Objectives. To perform a retrospective analysis of patients (pts) with SLL/CLL with emphasis on cytogenetic data and clinical outcome. Patients and Methods. We retrospectively reviewed our database of 201 SLL/CLL pts (registered between 1980 and 2010). Two pts were excluded because of the diagnosis of Monoclonal Benign Lymphocytosis (MBL). All patients registered between 1980 and 2010. Two pts were excluded because of the diagnosis of Monoclonal Benign Lymphocytosis (MBL). All these pts fulfilled the diagnostic criteria for CLL/SLL i.e. > 5x109/L peripheral blood lymphocytes co-expressing CD5/CD19, CD23 and low levels of surface immunoglobulin by flow cytometry (B-CLL pts) or a typical immunohistochemistry on lymphnode biopsy showing diffusely effaced nodal architecture with an infiltrate composed of mostly mature-appearing, small lymphocytes with <5x109/L lymphocytes (SLL pts). A bone marrow aspirate and/or biopsy was performed in all pts. Mantle cell lymphoma, hairy cell leukemia, follicular lymphoma or Richter syndrome were excluded. Caryotypic or FISH analysis was elected dose of 1.3 mg/m2 bortezomib. We present preliminary data on 23 evaluable pts for response: 12 have finished treatment (3-8 cycles, median of 6), of whom have of a CR and 3 patients achieved a partial response. These 3 patients were responding but had delays in recovery of platelet counts and either were removed from study as per protocol guidelines (2 pts, ages 52 and 54 years, both with MIPI score of 0) or progressed while awaiting recovery (1 pt, age 65 yrs old, MIPI score 5). At a median follow up of 5 months only this last patient has relapsed/progressed on the study. When combining Phase I and II pts who received a similar dose of bortezomib of 1.3 mg/m2 and completed therapy (20 patients), 100% of the patients have responded, 99% have achieved a CR, and only one CR progressed with a median follow up of 12 months of the combined group. Grade 3 toxicity was mainly hematologic, as expected, and one patient died from neutropenic infection with methicillin-resistant staphylococcus aureus bacteremia. She did not have prolonged neutropenia. Seven patients went off study due to delayed recovery of counts, usually after cycles 3-5, five of them after achieved a CR (currently all five remaining in CR). There was no grade 2-4 neutropenia. In conclusion, this phase II portion of the study demonstrates continued high rates of complete remission. The study is currently accruing and updated information will be presented at the time of the symposium.

0110
PHASE II-I STUDY OF BORTEZOMIB IN COMBINATION WITH R-HCVD AND R-MF IN TREATMENT-RESISTANT CYTARABINE (R-MA) IN UNTREATED MANTLE CELL LYMPHOMA (MCL)

Although 97% of patients with advanced MCL achieve a CR with R-HCVD/R-MA, patients still relapse over time, especially in those >65 years of age, who have a median time to failure free survival of 3 years. Bortezomib (B) has 31% single agent response rate in relapsed/refractory MCL and synergizes in vitro with many of the drugs in the above regimen. A phase I study of BR-HCVD/BR-MA did not show increased toxicity (Br J Haem. August 2010) and resulted in an elected dose of 1.3 mg/m2 bortezomib. We present preliminary data on the phase II study. Thirty-two patients have been entered, and their clinical presentation is shown in table 1.

Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>32</td>
</tr>
<tr>
<td>Median age (range)</td>
<td>64 (39-75)</td>
</tr>
<tr>
<td>Male</td>
<td>22 (69%)</td>
</tr>
<tr>
<td>Blastoïd variant</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Ann Arbor stage N</td>
<td>32 (100%)</td>
</tr>
<tr>
<td>Median 8 microglobulin</td>
<td>3 (1.59-9.7)</td>
</tr>
<tr>
<td>MIPI score</td>
<td>Low, Int, high, 37%, 44%, 19%</td>
</tr>
<tr>
<td>Ki-67 (17 pts)</td>
<td>&lt; 20, 21-40, &gt; 40 46%, 35%, 18%</td>
</tr>
</tbody>
</table>

All 23 evaluable pts for response have responded: 12 have finished treatment (3-8 cycles, median of 6), of whom 9 have achieved a CR and 3 patients achieved a partial response. These 3 patients were responding but had delays in recovery of platelet counts and either were removed from study as per protocol guidelines (2 pts, ages 52 and 54 years, both with MIPI score of 0) or progressed while awaiting recovery (1 pt, age 65 yrs old, MIPI score 5). At a median follow up of 5 months only this last patient has relapsed/progressed on the study. When combining Phase I and II pts who received a similar dose of bortezomib of 1.3 mg/m2 and completed therapy (20 patients), 100% of the patients have responded, 99% have achieved a CR, and only one CR progressed with a median follow up of 12 months of the combined group. Grade 3-4 toxicity was mainly hematologic, as expected, and one patient died from neutropenic infection with methicillin-resistant staphylococcus aureus bacteremia. She did not have prolonged neutropenia. Seven patients went off study due to delayed recovery of counts, usually after cycles 3-5, five of them after achieved a CR (currently all five remaining in CR). There was no grade 2-4 neutropenia. In conclusion, this phase II portion of the study demonstrates continued high rates of complete remission. The study is currently accruing and updated information will be presented at the time of the symposium.
(13q14.3) and the centromeric region of chromosome 12 (12p11.1-q11). Minimal data required were age, sex, Binet stage, lymphocyte count, presence of B symptoms, date of FISH analysis, percentage of cells with 17p deletion, time to first therapy (if required) and last follow-up visit. A significant proportion of patients also had information on IGHV status, CD38 and ZAP-70 expression and beta-microglobulin serum concentration. Results. We identified 108 (51%) patients with de novo and 60 (29%) patients with acquired 17p deletions (i.e. not present at CLL diagnosis). In 35 (17%) patients, FISH results were only available after therapy. Sixty-four percent were male and 36% female. Median age was 68 (range 22-99) years and Binet stage was B or C in 56% of patients. Additional FISH abnormalities were detected in 49% (13q-), 17p (+/-12) and 15% (11q-) of patients. CD38 and ZAP-70 expression was positive in 45% and 45% of patients, respectively, while the IGHV gene was unmutated in 66% of them. Median overall survival (OS) from FISH diagnosis was 37 months for the entire cohort. By univariate analysis, OS was significantly shorter in patients older than 65 years (P = 0.008), with B symptoms (P < 0.001), an acquired 17p deletion (P = 0.025), a beta-microglobulin concentration higher than 2.5 mg/l (P = 0.002), more than 20% of cells with deletion (P < 0.001) and Binet stage B or C (P = 0.002). Cox regression analysis revealed that four variables had independent prognostic value: Binet stage (P = 0.037), B symptoms (P = 0.005), age (P = 0.017) and percentage of cells with deletion (P = 0.016). The prognosis of CLL patients with a 17p deletion is modulated by simple and widely available clinical and laboratory features such as Binet stage and the presence of B symptoms. Patients with acquired deletions had a shorter median OS compared to those with de novo deletions (32 vs 39 months, P = 0.028), but only by univariate analysis. These factors could help clinicians when deciding the most appropriate therapeutic approach for an individual patient.

0112
TP53 MUTATIONS AND OUTCOME WITHIN THE CLL2O TRIAL OF THE GCLSG AND FGCLL/MW: AN INTERIM ANALYSIS

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The multicenter CLL2O trial aims at achieving a higher remission rate in “ultra-high risk”CLL [17p- and/or Fludarabine(F)- or Bendamustine (Benda)-refractory] by adding high-dose dexamethasone to alemtuzumab and prolongation of remission duration and survival by alemtuzumab maintenance or allogeneic stem cell transplantation (SCT). Ninety [F-Benda-refractory (n=40); 17p- without prior therapy (n=31) and 17p- in relapse (n=19)] enrolled pts were analysed for TP53 mutations. We used DHPLC to screen for TP53 mutations (Exons 4-10). Aberrant DHPLC profiles lead to sequencing of the respective amplicons. In addition, detailed characterization of prognostic markers and clinical features were performed. Median follow up time was 5.52 months. For 57 of the 90 patients median progression free survival (PFS) was 13.9 months and median overall survival (OS) was 17.7 months. No significant difference in PFS between the TP53 mutated and TP53 WT subgroup was detected (TP53 WT: 8.8 months vs. TP53 mutated: 13.9 months; p-value: 0.801). OS was similar in both subgroups with 18.7 months for TP53 WT and 17.7 months for TP53 mutated cases. In conclusion we show a high incidence of TP53 mutations in this “ultra-high risk”CLL cohort. This data, showing no significant difference between TP53 mutated and unmutated cases in clinical endpoints, suggests that the CLL2O treatment algorithm might be an effective strategy for TP53 mutated cases and can overcome their refractoriness to therapy. Refractory pts without detectable TP53 alterations are of importance for further studies.

0113
2SPONTANEOUS HEMATOLOGICAL REGRESSION IN CLL: LESSONS FROM THE ISRAELI CLL REGISTRY.

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Spontaneous regression in CLL is characterized by documented decrease in tumor burden not related to specific treatments. Cases of CLL regression have been occasionally documented. In the data of the Medical Research Council CLL trials, Thomas et al. reported a series of 10 patients who showed spontaneous remission out of 2570 (0.42%) registered cases (Br J Haematol, 2002, 116, 541). Recently Del Giudice et al. reported also the clinical and biological features (Blood, 2009, 114, 668) in 9 cases and found an overexpression of VH 3-30 and DICER gene by micro-RNA. An Editorial on this issue emphasized this ‘unfolding CLL mystery’ (Montserrat E). We analyzed the incidence of hematological remission in a large group of patients included in the Registry of the Israeli Study Group on CLL. Out of 1296 patients registered with CLL, 21 patients (1.6%) experienced spontaneous remission during the course of their disease. There were 10 women and 11 men, with median age at diagnosis of 69 years (range, 46-87). All but one were diagnosed in clinical Binet stage A, the remainder had en-
larged spleen compatible to stage Binet B. On flow cytometry, the median CD19/CD5 antigen coexpression on circulating CLL lymphocytes was 50%. Serum b2-microglobulin values were normal and mildly elevated in two patients; in these early stages no additional prognostic parameters were evaluated. The median follow-up period was 12 years (3-23 years). The regression was noted after a median of 8 years (range 1-22 years) of the follow-up. The maximal median lymphocyte count during the course of CLL was 15x10^9/l (9-75) and at regression dropped down to 2.3x10^9/l (1.2-4.7). The median CD19/CD5 antigen coexpression at regression was 28% (range, 17-57%). Distribution of T-cell subset during regression didn’t reveal significant changes. A possible association of this phenomenon in CLL with infection, smallpox vaccination or development of second malignancy has been previously suggested. In order to search associated diseases or events that may explain changes in the hematological condition, we reviewed medical reports of the patients. Eight patients suffered from recurrent infections, which required multiple hospitalizations and antibiotic treatments. One patient developed systemic lupus erythematosus with arthritis before therapy with verapamil. This drug by itself was found to induce apoptosis in CLL (Berrebi et al., Leukemia, 1994, 8, 2214). One patient developed the regression three years after being treated for autoimmune hemolytic anemia. Two patients had hypothyroidism treated with letrazol. Finally, three patients showed hematological improvement while developed new solid tumors (brain, lung and nasopharynx). In 7 patients no concomitant diseases at time of regression were documented. In conclusion, spontaneous remission in CLL is a rare phenomenon developed usually in early stage with no bad biological prognostic factors. Concurrent disorders such as infections and/ or second malignancy may contribute to decrease CLL clone.

0114
ANALYSES OF PARAMETERS WITH INFLUENCE ON RATE AND DURATION OF RESPONSE AFTER THE FIRST-LINE TREATMENT OF PATIENTS WITH HAIRY CELL LEUKEMIA
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Background. For many years the first line treatment for hairy cell leukemia (HCL) were splenectomy and alpha interferon (INF-α). Major breakthrough in the treatment of HCL has appeared with introduction of purine analogs, which improved the long-term outcome. It seems that the dilemma about the first line treatment was solved but there are still patients (pts) with HCL who need to receive more than one treatment lines. Additional research of biological parameters in HCL is needed to determine the optimal treatment strategy for this disorder.

0115
SERUM HSP 70 ANTIBODIES AS AN INDICATOR FOR TREATMENT IN CHRONIC LYMPHOCYTIC LEUKAEMIA PATIENTS
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Background. Chronic lymphocytic leukaemia (CLL) is the most common form of adult leukaemia in Western countries. The diagnosis is often accidental and there is no indication for early intervention. The current recommendation to start treatment includes disease-related symptoms, massive and/or progressive hepatosplenomegaly or lymphadenopathy, increasing bone marrow failure, autoimmune disease, and recurrent infections. The early treatment may be considered only in patients with unfavourable prognostic factors at the diagnosis. Heat shock proteins (HSPs) are molecular chaperones involved in a number of cellular functions in stress conditions. It is well established that leukemic cells may express HSPs including HSP70 on their surface. It has also been suggested that an increased surface expression of heat shock proteins on apoptotic tumour cells results in the generation of potent antitumour-T-cell responses. Anti HSP70 antibodies are known to play a role in immunological and neoplastic processes. However their significance in CLL has not been well documented. Aim: The aim of our study was the assessment of the anti HSP70 antibody concentration in the patients with CLL. Material and Methods. The studied group consists of 38 newly diagnosed CLL patients (aged 46-74), males and females in equal proportion. The patients were in A-C stages of CLL according to Binet scale. Twenty two of studied individuals required chemotherapy. Quantitative determination of anti-human HSP70 antibodies in the serum was done using commercial test (anti HSP70 Elisa kits, Stressgen). The results are presented as mean ± SEM. Statistical analysis was done using Shapiro-Wilk, Mann-Whitney and Spearman’s tests. Results. The levels of anti HSP70 antibodies were significantly lower in the group of patients that required chemotherapy in comparison to those in whom “watch and wait” strategy was applied (61.17±12.6ng/ml vs.118.86±27.8ng/ml; p<0.04). There was no association between the levels of antibodies and the stage of the disease. The study showed no correlations between anti HSP 70 antibody concentration and other parameters such as age, gender and some prognostic factors (LDH, β2-microglobulin). Conclusions. The assessment of low anti HSP70 antibody concentrations in CLL patients may support other clinical and laboratory features that indicate starting of chemotherapy in this cohort. Further studies are required to elucidate the role of anti HSP70 antibodies as a prognostic marker in CLL patients.
Chronic myeloid leukemia - Biology

**0116**

**EV1-REARRANGEMENTS AND HIGH EV1 EXPRESSION ARE FREQUENT IN CHRONIC MYELOID LEUKEMIA (CML) IN BLAST CRISIS (BC)***

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Blast crisis (BC) is the terminal phase of chronic myeloid leukemia (CML). Activation processes of oncogenic factors and/or mutations leading to loss of function of tumor suppressor genes in hematopoietic stem cells are involved in disease progression. MECOM (MDS1 and EV1 complex), located on chromosome band q26, functions as an oncogene by transcriptional regulation binding to DNA sequences in the promoter region of target genes and further interacts with epigenetic regulators, e.g. DNMT3A. An elevated EV1 expression is observed in myeloid malignancies with q26 rearrangements, some t(11q23)/MLL-rearrangements, monosomy 7/7q+, and also in a subset of acute myeloid leukemia (AML) with normal karyotype or other chromosomal abnormalities. Elevated EV1 expression is associated with poor clinical outcome. Moreover, elevated EV1 expression was found in patients with t(9;22)(q34;q11) and in t(11q23)/MLL rearranged AML. In BC-CML, EV1 expression was described as upregulated in patients with t(9;22)(q34;q11) (1% frequency; Grimwade et al. 2010). Here, we studied a cohort of 34 BC-CML patients (21 myeloid; 8 lymphoid; 4 not specified). All patients had been thoroughly characterized by cytogenetics and FISH to identify EV1-rearrangements. The proportion of EV1-rearrangements in BC-CML cohort (14%) was significantly higher in patients with t(9;22) compared to patients without rearrangement; median expression of EV1 was 6.2 fold increased in patients with t(9;22) (p=0.003). Interestingly, in both patients with lymphoid BC-CML a high EV1 expression with a ratio of 29.0 was detected. Subsequently, patients with detectable EV1 expression (22/30) were further investigated for possible t(11q23)/MLL-rearrangements using FISH. However, we did not detect any t(11q23)/MLL abnormality in this subgroup. In addition, WTI mutations were detected in 6/34 patients (17.6%). The frequency was significantly higher in patients with EV1-rearrangement (4/6; 50%) compared to those without (2/26; 7.7%; p=0.02). Of note, we sequenced ASXL1, CBL, CEBPA, IDH1, IDH2, IKZF1, KRAS, NRAS, NPM1, TET2, TP53 and investigated the BCR-ABL1 ratio for the complete cohort. Besides EV1-rearrangement no correlation between an elevated EV1 expression and any other molecular or cytogenetic marker was observed. Limited on cases without MLL- and EV1-rearrangements (n=26), the median EV1 expression in BC-CML patients was comparable to AML patients, based on a cohort of 260 AML cases we previously studied (mean 14.7 vs. 9.9; p=n.s.). Moreover, for patients with EV1-rearrangements (n=8) the EV1 expression in BC-CML is similar to patients harboring an AML with EV1-rearrangement (n=57) (mean 49.1 vs. 18.3; p=0.05). In conclusion, we demonstrated that EV1-rearrangements occurred with a high incidence of 23.5% and both translocation and overexpression of EV1 may play a central pathogenetic role in BC-CML. Further analyses are warranted to assess the clinical impact of these findings.

**0118**

**TRITERPENOID CDDO-ME IS HIGHLY SYNERGISTIC WITH THE HO-1 INHIBITOR SMA-ZNPP IN IMATINIB-RESISTANT CML CELLS***

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**Background.** In chronic myeloid leukemia (CML), resistance against one or more tyrosine kinase inhibitors (TKI) may develop during therapy, often because of BCR/ABL1 mutations. Novel therapeutic strategies and drugs are required to overcome TKI-resistance and to achieve long term remission in these patients. CDDO-Me is an oleandane triterpenoid that has been described to suppress survival of neoplastic cells in various tumors and leukemias by targeting several pro-survival factors including mTOR, Akt, and STAT5, and by inducing ROS-generation and thereby apoptosis. However, not all survival pathways may be suppressed by CDDO-Me. Notably, recent data suggest that exposure of leukemic cells to CDDO-Me results in a pronounced increase in heme-oxygenase-1 (HO-1), a major survival and resistance factor in CML.  **Aims.** The aim of this study was to explore whether CDDO-Me exerts effects on imatinib-resistant leukemic cells carrying BCR/ABL1 mutations, and to learn whether the HO-1 inhibitor SMA-ZnPP would synergize with CDDO-Me in producing growth inhibition and apoptosis in CML cells.  **Methods and Results.** As assessed by 3H-thymidine uptake, CDDO-Me was found to inhibit the proliferation of the CML cell line K562 as well as BaF3 cells transfected with different BCR/ABL1 (T315I, E255K, Y253F) mutants and their intracellular nuclear localization was determined by IE BaF3 cells expressing different BCR-ABL1 mutants were cultured in liquid media (in the absence of IL-3) or in soft agar to investigate their transforming ability. The proliferative potential of each BCR-ABL construct was also ascertained by "ex vivo" after lentiviral expression in CD34-positive cells. Results: Both NLSs directed GFP in the nuclear compartment, while the NES favored its cytoplasmic localization. The latter effect was abolished after treatment with the exportin-1 inhibitor Leptomycin B (LMB). Despite these findings, wild-type BCR was an exclusively cytoplasmic protein because of a lack of nuclear import. However, removal of the N-terminus of the nuclear localization signal (NLS) of the C-terminal Protein Kinase C conserved region 2 (DC2) and of the Rho-GAP domain generated a construct displaying strong nuclear staining that was further increased by LMB. As the OD and part of the DC2 are preserved in BCR-ABL, we wanted to establish if these domains contributed to the subcellular localization of the oncprotein. To this end, we generated a ADO-AD2/CML mutant and its kinase-proficient counterpart ADO-AD2/CML. However, ADO-AD2/CML does not display transforming potential, suggesting that the DC2 domain plays a pivotal role for BCR-ABL-dependent oncogenic activity.
patients who had developed resistance against two or more TKI. CDDO-Me was found to inhibit proliferation of leukemic cells in all patients tested, with IC50 values ranging between 0.1 and 0.5 µM. No differences in IC50 values were observed between TKI-naive and TKI-resistant CML cells. Since BCR/ABL-targeting TKI or other drugs, when used as single agents, usually fail to induce long-term remission in advanced disease, drug combinations are currently being tested preclinically and in clinical trials. In this study, we applied the combination CDDO-Me+SMA-ZnPP and found that this combination is highly synergistic in producing growth inhibition in K562 cells and primary CML cells isolated from imatinib-naïve and imatinib-resistant patients, including one patient in whom BCR/ABL T315I was detected. We also examined whether CDDO-Me would exert synergistic effects on CML cells when combined with BCR/ABL TKI. We therefore applied the combinations CDDO-Me+dasatinib and CDDO-Me+nilotinib in K562 cells. Both combinations were found to synergize in producing growth inhibition. Conclusions. CDDO-Me inhibits the proliferation of imatinib-resistant BCR/ABL+ cell lines and of primary CML cells isolated from untreated and TKI-resistant patients, including cells carrying the BCR/ABL mutant T315I. Our data also show that CDDO-Me and BCR/ABL TKI synergize in producing growth inhibition in CML cells. Synergistic drug interactions were also observed with CDDO-Me+SMA-ZnPP which may be explained by the HO-1-inducing effect of CDDO-Me.

0119
IS P210 PHILADELPHIA POSITIVE-ACTIVE LYMPHOBLASTIC LEUKEMIA A LYMPHOBLASTIC CRISIS OF LATENT CHRONIC MYELOID LEUKEMIA?

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Background. The Philadelphia (Ph) chromosome is a hallmark cytogenetic abnormality in chronic myeloid leukemia (CML), and is also present in ~25% of adult acute lymphoblastic leukemia (ALL). Although the p210 BCR/ABL isomform is predominant among CML patients, about 30% of adult Ph+ ALL have p210 and the remainder harbor the p190isoform. If left untreated, CML invariably progresses to a blast crisis, which is typically of myeloid lineage. However, in ~30% of CML cases the blasts are of lymphoid lineage. In the absence of documented chronic phase CML, this lymphoblastic presentation poses a diagnostic challenge to differentiate from de novo Ph+ ALL. Neutrophil-FISH (N-FISH) using peripheral blood cells for CML diagnosis and/or to monitor imatinib response is widely available in Japan. Importantly, N-FISH also identifies the Ph chromosome lineage to neutrophils in some patients with ALL. Aims and Methods. To assess the clinical differences between p210- and p190-ALL, a retrospective chart review was performed for cases of Ph+ ALL in the Tohoku Hematology Forum study group between October 2000 and September 2010. Eighty-four Japanese patients with Ph+ ALL were enrolled in this study. The data reviewed included diagnosis, treatment, hematologic parameters, cytogenetic characteristics, and clinical outcome. N-FISH was performed at the time of diagnosis in 24 cases (p210, n=11; p190, n=13). Results. No definite clinical differences were identified between p210-ALL (n=25) and p190-ALL (n=56). However, similar to what is observed in CML, N-FISH identified BCR-ABL positive neutrophils in 91% (10 of 11) of p210-ALL (average 74.4%). In contrast, BCR-ABL positive neutrophils were only found in 38% (5 of 13) of p190-ALL (average 61.3%) which was statistically different than p210-ALL (p=0.008 by Fisher’s test). All p210-ALL cases were then subdivided based on N-FISH results: secondary ALL, with Ph chromosome myeloid lineage involvement (n=15); and “de novo ALL”, without myeloid lineage involvement (n=9). Using this classification, additional features were identified that significantly differed between the two subgroups (secondary ALL vs. de novo ALL); laboratory parameters (platelets: 95x103/µl vs. 26x103/µl, p=0.005; basophils: 0.56% vs. 0%, p=0.041), immunophenotypes (CD13+: 12/15 vs. 2/9, p=0.005; CD34+: 11/15 vs. 1/9, p=0.005), and percentage of residual normal clones (6/15 vs. 8/8, p=0.005). Conclusion. N-FISH revealed myeloid lineage involvement in “secondary ALL”, which was largely comprised of p210-ALL cases. As this combination resembles CML in several other clinical features, these data suggest that “secondary ALL” may represent CML presenting in lymphoblastic crisis. During a latent phase prior to the clinical onset of CML, Ph chromosome patients could acquire genetic abnormalities to transform to lymphoblastic crisis. Despite the great advances in CML treatment with the development of imatinib and other tyrosine kinase inhibitors, the latent phase of CML is still poorly understood and may have the potential to progress to p210-ALL in addition to clinically overt CML.

0120
REGULATION OF CELL CYCLE IN PRIMITIVE CHRONIC MYELOID LEUKAEMIA (CML) VS NORMAL STEM CELLS

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Background. Chronic myeloid leukaemia (CML) is a clonal haemopoietic stem cell (HSC) disorder associated with the Ph chromosome and BCR-ABL oncogene, encoding a constitutively active tyrosine kinase. Aims. We hypothesise that the resistance of CML stem cells to chemotherapy is dependent on pathways that are deregulated in malignancy, offering the possibility of developing therapeutic approaches that are selective for leukaemic versus (vs) normal HSC. Methods. We have investigated the transcriptional differences between quiescent (G0) normal and CML stem cells by performing RNA and miRNA profiling. Biochemical analysis was carried out by flow cytometry and western blotting. Results. G0 CD34+ haemopoietic cells were isolated from normal controls and patient samples and gene profiling revealed differences in the expression of cell cycle genes, including CDC20, CHEK1, CDK9, CDC27, CDC2, CDC6 and Cyclin B2. Interestingly, E2F1, the communal transcription factor for all the genes we found differentially expressed, was abnormally regulated in CML and it is likely that, together with is regulator Rb, E2F1 is a key player in modulating CML stem cell quiescence. E2F1 was upregulated in CML CD34+ cells compared to normal, at both the protein and mRNA level. Furthermore, its activation in these cells was confirmed by the high level of phosphorylated Rb. To understand if miRNAs have a role in CML stem cell survival and in particular in the regulation of the E2F1/Rb pathway, we carried out miRNA arrays. CD34+ cells isolated from normal and patient samples were used to perform genome-wide miRNA expression profiling (LC Sciences www.lcsiences.com). Initial data analysis has provided several potential candidates which are under investigation. Summary/Conclusions. These data suggest that the E2F1/Rb signalling pathway may have a role in CML stem/progenitor cells cycle status and contribute to the understanding of CML stem cell quiescence, suggesting new strategies to target CML stem/progenitor cells by preventing or reversing these effects. In addition, enhanced knowledge of the survival pathways that are operative within CML stem cells, which may lead to eradication of this critical population, would be of relevance to other forms of cancer.
REDUCED EXPRESSION OF THE LTB4 MEMBRANE RECEPTOR BLT1 EXPLAINS ALOX5 DOWN-REGULATION IN NEWLY DIAGNOSED CML PATIENTS

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Background. Alox5 has been reported to be upregulated in mouse leukaemic stem cells (LSC), and this is not inhibited by imatinib. Furthermore, mice transplanted with Alox5 deficient BCR-ABL1+ bone marrow cells were resistant to the induction of chronic myeloid leukaemia (CML) (1). Alox5 deficiency was found to have no effect on BCR-ABL-negative cells. This suggests that Alox5 is important in LSC growth and is essential for the development of CML. To investigate the role of Alox5 in imatinib treated CML patients. Methods: Functional Alox5 was assessed using an LTB4 ELISA and Alox5 mRNA determined via a TaqMan gene expression assay. BLT1 protein levels were assessed by cell surface FACS analysis. Results. Alox5 expression was measured in 27 chronic phase patients at diagnosis and at 3, 6 and 12 months following imatinib treatment. At diagnosis Alox5 expression in all patients was below that seen in samples from normal controls. On treatment initiation Alox5 levels increased to normal values in all patients whose disease was controlled to progress to chronic phase (BC) where levels remained low. All increases in Alox5 levels were statistically significant, diagnosis and 3 months (p=0.003), 6 months (p=0.001), and 12 months (p=0.024) in patients who subsequently achieve a CCR. Plasma LT4 levels were utilised as a measure of Alox5 function. In all CML patients LT4 levels were higher than that measured in samples from normal controls, and all patient levels increased further on imatinib treatment. Levels in BC were always higher compared to chronic phase. These data in clinical material are in conflict with previously published work utilising a mouse model, since we find that LT4 levels are increased despite low Alox5 expression. To investigate further we undertook determination of LT4 receptor (BLT1) levels. BLT1 surface expression was found to be extremely low in newly diagnosed chronic phase CML patients. BLT1 protein in newly diagnosed patients was significantly lower than the level observed in patients responding to tyrosine kinase inhibitor (TKI) treatment (defined as a BCR-ABL1 ratio 1-10%) and those patients who achieved a CCR (p=0.004 and p<0.0001 respectively). Conclusion. Alox5 expression is low and LT4B levels are high in CML patients at diagnosis. This is likely the result of increased negative regulation of Alox5 via the arachidonic acid pathway intermediates (5-HEPTE and 5-HEPGE) and a lack of positive feedback through LT4 due to the reduced expression of the BLT1 receptor. Clearly different pathways of Alox5 dysregulation exist between the CML mouse model and patients which should be considered when translating findings from the mouse model to a clinical setting.

DYSREGULATION OF THE TUMOUR SUPPRESSOR PTEN IN CHRONIC MYELOID LEUKAEMIA

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Background. Chronic Myeloid Leukaemia (CML) is a myeloproliferative disorder characterised by the BCR-ABL1 fusion oncogene. Despite the uniformity of causation and therapeutic modality (Imatinib), patient heterogeneity in their response to imatinib therapy (Phosphatase and Tensin Homolog) is a tumour suppressor gene, which codes for a phosphatase antagonist of the PI3K/Akt mitogenic axis. The mechanism of repression is yet to be fully elucidated, but both promoter hypermethylation and dysregulation of the pseudogene PTENP1, which acts as a decoy for suppressive microRNAs, have been implicated. Aims. To investigate whether CpG hypermethylation and/or changes in PTENP1 pseudogene expression contribute to the down-regulation of PTEN in CML, and to correlate expression with patient Sokal score and 12month clinical response. Methods. Peripheral blood lysates from 32 chronic-phase CML patients at diagnosis and 30 normal volunteers were used to isolate DNA and mRNA. The DNA was bisulphite treated and used for High Resolution Melt analysis (HRM) and Pyrosequencing to detect methylation studies. Two CpG island were interrogated for methylation: one residing in the 5' UTR of PTEN by HRM, and the other 1.4kb upsteam of exon 1 by Pyrosequencing. The mRNA was converted to cDNA and used for qPCR gene expression studies. The qPCR employed primers specific for PTEN and PTENP1, with B2M as the endogenous control. Expression levels were given as delta-CT relative expression ratios (RE). 16 CML samples were classified by Sokal score (low, intermediate & high risk) and by 12mth response to Imatinib (400mg/day), defined by attainment of complete cytogenetic response (CCyR). Results. PTEN was shown to be significantly under-expressed in CML compared with Normal samples (median delta-CT 0.51 and 1.69, respectively; p=0.001). PTENP1 was conversely expressed (RE 2.06 and 0.62; p=0.0013). Patients with a high Sokal score had a significantly higher PTEN expression than those in the low risk group (1.04 and 0.52, respectively; p<0.05), but there was no difference for PTENP1. There was also no significant difference in expression for either gene between the 12mth optimal and suboptimal response groups. Neither HRM nor pyrosequencing showed any evidence of CpG hypermethylation in any of the patient samples. Summary/Conclusions. Although loss of PTEN is known to be a marker of disease progression in many tumour types, in CML the dynamic appears to be more complex. It was consistently down-regulated in diagnostic CML, weakening the case for its role as a decoy for suppressive microRNA as a mechanism in PTEN dysregulation. The epigenetic assays performed found no evidence for promoter hypermethylation, suggesting that PTEN down-regulation in CML occurs via a yet to be elucidated mechanism.
PROTEIN SIGNALLING TRIGGERED BY IMATINIB AND DASATINIB IN THE MYELOID BLASTIC CRISIS OF CML: AN IN VITRO PROTEOMIC AND PHOSPHOPROTEOMIC STUDY

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Background. Imatinib resistance (IM-R) developing in approximately 30% of Chronic Myeloid Leukemia (CML) patients (pts) recognizes both BCR-ABL-dependent and BCR-ABL-independent mechanisms. The former include point mutations in the BCR-ABL kinase domain and amplification of the BCR-ABL gene locus while the latter are still poorly understood, probably involving improper activation/inactivation of alternative signaling pathways. Aims. We describe preliminary results of a study employing immortalized CML cells - either sensitive or resistant to IM - to perform Reverse Phase Protein Micro-Arrays (RPMA) aimed at characterizing the proteomic profiles of these cell lines and at identifying BCR-ABL-independent pathways that contribute to the development of IM-resistant CML. Methods. RPMA is a high-throughput system for protein signaling pathway profiling. The phosphorylation state of kinase-associated therapeutic targets provides direct information regarding the target and off-target effects of different drug treatments. Human CML cell lines, sensitive (K562S, LAMA43S) and resistant (K562R, LAMA43R) to Imatinib, were incubated with IM 1μM, Dasatinib (DS) 1μM or LY-294002 10μM (LY) used as a control. After 2 or 12 hours, cells were placed in a preservation that suppresses fluctuations in kinase pathway proteins. RPMA were used to quantitatively map 45 cell signaling pathway endpoints, including autophagy, DNA repair systems, DNA damage and transcriptional factors crucial for CML. Results. Previous evidence has demonstrated that K562R are unresponsive to IM because of unknown BCR-ABL-independent mechanisms, while LAMA43R display BCR-ABL genomic amplification. Compared to their sensitive counterpart, K562R exhibited a paradoxical reduction in BCR-ABL signaling at BCR(Y177), CRKL(Y207), ERK(T202/Y204) and Cofilin(D39), associated with over-expression of autophagy markers (ATG5 and LC3B). Conversely, LAMA43R presented increased BCR-ABL signaling at BCR(Y177), CRKL(Y207) and ERK(T202/Y204). In drug treatment endpoints, DS was confirmed stronger than IM in abrogating BCR-ABL auto-phosphorylation on BCR(Y177). We found HDAC3 induction in both K562R and LAMA43R and this event was positively associated with increased expression of c-MYC and NUMB and higher phosphorylation on mTOR(S2445), pAkt(S606), CHK1(S345), FOXO1(T24), FOXO3a(T32), but not on BCR(Y177) or c-ABL(Y245). Conclusions. Taken together, our data confirm the value of the RPMA assay to investigate improperly activated pathways that could identify one or more potential targets for future treatment strategies of IM-resistant pts.

STATINS CAN POTENTIATE ANTILEUKEMIC EFFECT OF IMATINIB THROUGH THE INHIBITION OF ABCB1 AND ABCG2 ACTIVITY

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Background. Despite clinical success of imatinib in the majority of patients, development of resistance becomes relevant clinical problem. Variations of intracellular concentration of tyrosine kinase inhibitors, dependent on the activity of drug transporters, are among major factors responsible for development of resistance in the absence of BCR/ABL mutations. Recent data support the idea that resistance results from the balance of drug influx mediated mainly by OCT1 transporter and drug efflux mediated by ABC transporters. According to the recent knowledge, in clinical settings imatinib is a substrate of ABCB1 and ABCG2 transporters. Strong evidence suggest that agents modulating membrane cholesterol level, e.g. statins, are able to change conformation of membrane-bound drug transporters and increase intracellular imatinib concentration. Aim of the study. Potentiation of antileukemic activity of imatinib in cell lines transformed with BCR-ABL1 oncoprotein and primary human CML CD34+ cells employing statins. Methods. The following cell lines were used in the study: primary CD34+ cells obtained after informed consent from the patients with CML as well as from healthy blood donors; cell lines: K-562, 32Dcl3 wild type and BCR/ABL-transformed. ABC transporters activity was evaluated using specific fluorescent substrates: BODIPY-prazosin (for ABCG2) and rhodamine 123 (for ABCB1) while direct imatinib efflux studies were performed using 14C-labelled drug. Imatinib-mediated cytotoxicity was analyzed using cell viability and apoptosis assays (XTT and propidium iodide staining). Clonogenic assay after drug treatment was performed. Expression of BCR-ABL-dependent proteins was assessed. Results. Cytochemical analysis of ABC transporters activity has shown that lovastatin significantly decreased the activity of ABCB1 and ABCG2-mediated efflux capacity. The effect was completely reversed by the addition of cholesterol. It was observed both for stable cell lines and primary CD34+ CML cells. These results were confirmed using radiolabeled 14C-imatinib concentration measurement after incubations with
and 139 CML patients, including 106 chronic phase (CP), 13 accelerated phase (AP) and 20 blast crisis (BC) samples, which showed no expressions of these 2 proteins, leukemic cell lines revealed an obvious up-regulation of B23 and C23. Moreover, compared to wild-type counterparts and cells obtained from patients clinically resistant to imatinib with no detectable ABL kinase domain mutations (Figure 1). We did not observe changes in initial influx of the drug between statin-treated and control cells. Interestingly, statins did not influence the expression of ABC transporters. Lovastatin enhanced cytotoxicity of imatinib in BCR/ABL-positive cell lines and CML CD34+ primacells as compared to primary AML patients. No NPM1 mutations were found from 20 CML samples (10 B23+ and 10 B23-), which were not consistent to B23 protein expressions. B23 and C23 positivity significantly correlated with shorter OS and poorer prognosis. One patient became B23 positive as disease progressed from CP to BC. Conclusions. It could be concluded that B23 and C23 may be involved in resistance to imatinib and be useful in predicting progression of CML. Further investigation is needed to explore their involved mechanisms in progression of CML.

**0128**

### THE ROLE OF SPARC IN CHRONIC MYELOID LEUKEMIA

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Background. Secreted protein acid and rich in cysteine (SPARC/os-teonectin/BM-40) is a multifunctional matricellular glycoprotein with counter-adhesive properties and effects on cell shape, proliferation, cell cycle and angiogenesis. In some types of cancer, SPARC correlates with poor prognosis (melanoma, glioma, prostate and breast cancer), while in others the protein functions as a tumor suppressor (ovarian, pancreatic and colorectal cancers). Studies of SPARC in hematopoietic malignancies resulted in conflicting reports about its role as a tumor suppressor (5q- MDS and AML with rearrangement of the MLL gene) or promoter (multiple myeloma and plasmacytoma). In chronic myeloid leukemia (CML) a recent study indicates that intracellular SPARC may be involved in resistance to imatinib (IM) Aims. Inhibition of exogenous SPARC for 2 days and then with IM for 24 h. Cell proliferation was evaluated after 72 h by ATP-lite: a cytotoxicity of 18±3,2% and 29±1,6% vs untreated cells was recorded with SPARC and IM alone respectively; their association induced a reduction of cell viability of 37,5±3,7%. After 96 h the combination SPARC/IM resulted more effective than IM alone with an increase of cytotoxicity of 16,5±3% (p<0.01 vs IM alone). Flow cytometry analysis revealed an accumulation of K562 cells in G0/G1 after 24 h exposition to IM alone (15±1,7% vs untreated cells; p<0.01) or SPARC alone (14,5±4,1%; p<0.01) while SPARC/IM combination showed an additive effect (26,5±5,3%; p<0.001). Conclusion. SPARC production is downregulated in CML cells. Treatment of IM induces overproduction of the tumor suppressor (tumor suppressor pathway) in these cells obtained from patients clinically resistant to imatinib with no detectable ABL kinase domain mutations. The combination SPARC/IM results in a significant reduction of cell viability of 37,5±3,7%.

**0127**

### OVER-EXPRESSED NUCLEOPHOSMIN/ B23 AND NUCLEOLIN C23 PREDICTED DISEASE PROGRESSION IN CHRONIC MYELOID LEUKEMIA

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Background and Aims. Nucleophosmin (NPM1) and nucleolin (C23), are multifunctional nucleolar proteins and play important role in various aspects of ribosome biogenesis from transcription regulation to the assembly of pre-ribosomal particles. Compared to C23, NPM1 is much more investigated for its relation to cancer. The deletion and translocation of NPM gene have been observed in hematological malignancies. NPM1 mutations occur specifically in about 80% of adult de novo AML. However, NPM1 mutation and B23 protein expression were rarely reported in CML. Our previous study showed that proteins B23 and C23 were over-expressed in relapsed/refractory acute leukemia patients. Methods. In order to investigate their clinical significances in CML, B23 and C23 expressions were detected by western blot and RT-PCR in leukemia cell lines K562, K562/ADR (adriamycin-resistant K562), KG1 (imatinib-resistant K562) and 139 CML patients, including 106 chronic phase (CP), 13 accelerated phase (AP) and 20 blast crisis (BC). Results. Compared to healthy control samples, which showed no expressions of these 2 proteins, leukemic cell lines revealed an obvious up-regulation of B23 and C23. Moreover, significantly higher expressions of B23 and C23 were found in 2 resistant cell lines compared to the parental K562 cells. In primary CML samples, much higher expressions of B23 and C23 were noted in CML-BC than in CML-AP and CML-CP, which were 75±(15/20), 7.7±(1/15) and 12.3±(13/106) respectively. The concomitant expression of B23 and C23, both positive or negative, was noted in 58% (13/23) patients. No NPM1 mutations were found from 20 CML samples (10 B23+ and 10 B23-), which were not consistent to B23 protein expressions. B23 and C23 positivity significantly correlated with shorter OS and poorer prognosis. Results show that B23 and C23 over-expressed in CML cells. Treatment of IM induces overproduction of the tumor suppressor (tumor suppressor pathway) in these cells obtained from patients clinically resistant to imatinib with no detectable ABL kinase domain mutations. Further investigation is needed to explore their involved mechanisms in progression of CML.

**0129**

### IMATINIB HAS THE POTENTIAL TO EXERT ITS ANTILEUKEMIA EFFECTS BY DOWN-REGULATING HERG K+ CHANNELS IN CHRONIC MYELOGENOUS LEUKEMIA

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Background. Imatinib (STI571, Gleevec), a powerful protein tyrosine kinase (PTK) inhibitor, is the current first-line therapy for all newly diagnosed chronic myeloid leukemia (CML). Studies aimed at deci-
phering the mechanism of imatinib anti-leukemic activity have focused on inhibited PTKs activity, such as Abl, c-Kit, and the platelet-derived growth factor receptors. Indeed, in addition to the mediation of cellular events such as cell proliferation, differentiation, embryonic development, metabolism, and oncogenesis, PTKs also regulate a large number of ion channels. Increasing evidence suggests that PTK inhibition affects the activity of a large family of voltage-dependent ion channels. Aims. hERG1 K+ channels are highly expressed in leukemia cells and appear of exceptional importance in favoring leukemogenesis, while the effect of imatinib on hERG1 K+ channels in CML has not been well characterized. The present study explored a possible regulatory effect of imatinib upon hERG1 K+ channels as a mean to uncover the mechanisms involved in the antileukemia activity of this PTK inhibitor in CML. Methods. Real-time PCR, flow cytometry analysis and the whole-cell patch clamp technique were used to analyze effects of imatinib on hERG1 K+ channels. The hERG1 K+ channel inhibitor was used to detect whether hERG1 K+ channels act a potential target for imatinib-induced antileukemia effects in primary CML cells and Bcr-Abl-positive K562 cells. Results. Present data demonstrated that hERG1 was highly detected in K562 cells and primary CML cells. K562 cells cultured with imatinib (5 μM), the expression of hERG1 mRNA and protein were down-regulated by (67.9 ± 19.2) % and (64.8 ± 12.4) %, separately. Furthermore, current recordings of the hERG2/3T cells transiently transfected with hERG showed that imatinib markedly reduced the step hERG currents amplitude to (45.7 ± 2.5) % (n = 6) of control at 10 mV, and peak tail currents amplitude to (25.8 ± 3.1) % (n = 6) of control at 20 mV. Unexpected, imatinib failed to alter the voltage-dependence of hERG channel activation. The Old physiologic effects of suppressing hERG1 K+ channels by imatinib illustrated that cells pretreated with imatinib in the presence of 1 μM E-4031, a specific hERG1 K+ channels inhibitor, which achieved 1.9-fold higher the inhibition of proliferation and 1.3-fold stronger induction of apoptosis than that of cells treated with imatinib. Moreover, the synergistic effects of E-4031 and imatinib on the suppression VEGF secretion and NF-κB activation were observed. When treated with imatinib in the presence of E-4031, the expression of VEGF mRNA was significantly reduced by 60%. The mean VEGF concentration in culture supernatants from cells treated with imatinib combined with E-4031 was 199.9 ± 101.5 ng/L per 106 cells, which was 1.6 times higher inhibition efficiency than that of cells treated with imatinib. Conclusions. Our data demonstrate that imatinib down-regulates hERG1 K+ channels currents, which involves in imatinib-induced antileukemia effects in CML. These findings reveal a novel potential molecular mechanism of antileukemic activities by imatinib, which independent of targeting on the Bcr-Abl pathway.

**0130**

OVEREXPRESSON OF FBPI IS ASSOCIATED TO HIGH Sokal RISK IN CHRONIC MYELOID LEUKEMIA PATIENTS

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Background. The oncogenic transformation of Chronic Myeloid Leukemia (CML) cell of origin has been associated to an increased glucose metabolism, while the glycolytic pyruvate is directed away from the mitochondria, converted into lactate and secreted from the cell. This metabolic conversion is termed aerobic glycolysis, or Warburg effect and very essentially, it serves to support the tumor cell proliferation, by providing nucleotides, aminoacids and lipids enough to replicate all of cellular content. CML patients are historically stratified according to their Sokal risk score, which for more than 30 years has been regarded as the most significant prognostic factor in this hematological malignancies. The putative genetic and/or genomic basis driving this stratification are still not known. Aim. Here we present data obtained from GEP experiments aimed at the identification of genes and pathways able to predict and/or to elucidate the onset of the disease and the disease course in C-P-CML pts by analyzing the transcriptome of the CD34+ cell fractions obtained at diagnosis from a cohort of high and non-high Sokal risk CML patients. Patients and Methods. Overall, 67 patients with previously untreated CML in chronic phase (CP) entered the study; all of them have been enrolled in GIMEMA CML protocols and provided highly enriched CD34+ cell fractions from peripheral blood. Gene expression profiling (GEP) was performed, in order to identify genes most significantly and most differentially expressed between CML patients and healthy controls. All of them were analyzed in real-time experiments, in order to validate the GEP data. Results. By GEP, 82 probe-sets, corresponding to 78 genes resulted significantly differentially expressed between high and non-high Sokal risk patients in a supervised analysis of gene profiles. A gene enrichment analysis of this profile showed that genes involved in hyoxia and oxidative stress resulted significantly over-expressed in the comparison between high and non-high risk patients. Particularly interesting resulted a significantly higher expression in high Sokal risk patients of FBPI (fructose-1,6 bisphosphatase), a key-enzyme of gluconeogenesis, together with a significant over-expression of genes coding for enzyme involved in gluathione biosynthesis (FBP1, GSTM4, SEPP1). These data suggest that CD34+ cells obtained from high Sokal risk patients might exhibit an unexpected moderate of the glycolytic flux, mainly due to the over-expression of FBPI, which might cause a re-direction of the pathway into the pentose phosphate shunt. Moreover, we validated by Real-time the de-regulated expression of these genes in a different set of CML patients, thus confirming that they are differentially expressed between high and non-high risk patients and we found that HIF1α, which is a gene that may be involved in the metabolic reprogramming, is indeed deregulated in our system. Overall, our data demonstrate for the first time, that the expression at diagnosis of sugar metabolic enzymes, might drive the evolutive Sokal risk of CML patients.

**0131**

EVI-1 GENE OVER-EXPRESSION: A POSSIBLE MECHANISM OF THROMBOCYTOYSIS AT CHRONIC MYELOID LEUKEMIA ONSET

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Background. A considerable proportion of chronic myelogenous leukemia (CML) patients are characterized by elevated platelet counts at diagnosis. The underlying mechanisms are still poorly understood. Besides, thrombocytosis in acute leukemias has been recently linked with structural abnormalities of the short arm of chromosome 3, where EVI-1 gene maps. Activation of the EVI-1 oncogene has been reported in acute myeloid leukemia, CML in blast crisis, and less common in chronic phase-CML patients. The aim of this study was to investigate the expression of EVI-1 in chronic phase (CP) CML patients with extreme thrombocytosis in comparison to patients with normal or moderately elevated values as well as to patients with essential thrombocytemia (ET). Materials and Methods. In total, 21 patients with BCR-ABL-positive CP-CML and platelet counts above 1000x10⁹/L (ranging 1192.9±542.4x10⁹/L vs 708.1±551.9x10⁹/L; Pearson Chi square, p=0.006) were studied for EVI-1 over-expression by reverse transcription polymerase chain reaction (RT-PCR) and the results were compared to a group of 22 BCR-ABL-positive CP-CML patients with platelet counts ranging 209-535x10⁹/L. A group of 15 patients with ET, including 6 cases positive for JAK2 V617F mutation, were also included. Major clinical and laboratory variables, as well as the BCR-ABL type of transcripts were analysed. Results. In total, 21 patients with CP-CML were found to be EVI-1(+). The incidence of EVI-1 over-expression was significantly higher in CML patients with thrombocytemia >1000x10⁹/L where it was found in 16/21 cases (76.2%) compared to 5/22 (22.7%) in CML patients with lower platelet counts (Fisher’s Exact Test, p=0.001). No significant correlation between EVI-1 gene status and basic laboratory features such as leukocyte counts, hemoglobin levels, and age nor with the BCR-ABL type of transcripts was found. However, even in the whole CP-CML group, the platelet counts in EVI-1(+) patients was significantly higher compared to the EVI-1(-) cases (mean 1192.9±542.4x10⁹/L vs 708.1±551.9x10⁹/L; Pearson Chi square, p=0.006). Interestingly, none of the 15 ET patients (0%) had detectable EVI-1 mRNA levels. Conclusions. Until recently the EVI-1 gene over-expression was considered as a marker of disease progression. Up to our knowledge the present study provides for the first time additional evidence that the oncogene might play a role in the pathogenetic mechanisms of thrombocytosis in CML patients that differ from the involved pathways in ET.

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DISTINCTIVE MIRNA EXPRESSION PROFILE AND FUNCTIONAL ROLE IN K562 CELLS

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Background/Aims. MicroRNAs (miRNAs) are short non-coding regulatory RNAs that control gene expression at the post-transcriptional level. Deregulation of miRNA expression has been discovered in a variety of tumors and it is now clear that they contribute to cancer development and progression. Chronic myeloid leukemia (CML) is an extensively studied neoplasms. The hallmark of CML is the Philadelphia chromosome, created by the t(9;22)(q34;q11) translocation, resulting in the formation of the bcr-abl oncogene, which codes for a deregulated tyrosine kinase. Imatinib, a bcr-abl inhibitor, selectively induces apoptosis of Bcr-Abl+ cells and is successful in treating CML patients. One major obstacle to imatinib-based therapies is imatinib resistance. In an attempt to override resistance, second-generation inhibitors, such as dasatinib were developed. Dasatinib is a multikinase inhibitor targeting bcr-abl and Src kinases and inhibiting most imatinib-resistant mutants. Our objective is to decipher an miRNA expression signature associated with CML and to determine potential target genes and signaling pathways affected by these signature miRNAs.

Methods. Global miRNA expression profiling was performed with miRNA-based microarrays. Analysis was performed using Partek Genomics Suite and validation was done by Taqman-miRNA. Bioinformatics was performed using: Target Scan, Ingenuity Pathway Analysis, KEGG pathway database. Results. We analyzed the miRNAs expression profile of K562 cells in reference to a pool of healthy blood. We also looked into the expression profile of K562 cells treated with imatinib or dasatinib. With the aid of unsupervised hierarchical clustering we found that healthy blood samples were clustered separately from K562 cells. Untreated K562 cells were clustered separately from treated ones and imatinib treated cells were clustered closely to those treated with dasatinib. Seventy-five miRNAs were downregulated and 55 upregulated in K562 cells as compared to healthy blood. Seventeen miRNAs were upregulated and 21 were downregulated following imatinib or dasatinib treatments. Following miRNA real-time PCR validation, we focused on 8 statistically significant differentially expressed miRNAs: 7 were downregulated (miR-31, miR-34a, miR-143, miR-155, miR-196b, miR-564) and one was upregulated (miR-128) in K562 cells. MI-R-128 was downregulated following imatinib and dasatinib treatments. MiR-564 was upregulation following imatinib treatment. The expression of the remaining 6 miRNAs was not altered following either drug treatment. We next analyzed predicted targets and affected pathways of the 8 deregulated miRNAs using Target Scan and Ingenuity Pathway Analysis. Reassuringly, the analysis identified cancer, and specifically CML, as the main disease associated with these 8 miRNAs. MAPK, TGF-β and Wnt were the main molecular signaling pathways related with these expression patterns. Utilizing Venn diagrams we found appreciable overlap between the CML-related miRNAs and the MAPK and TGF-β-related miRNAs. Conclusions. Our data suggest that our identified miRNAs might offer a pivotal role in CML. Nevertheless, while these data point to a central disease, the precise molecular pathways targeted by these miRNAs is variable implying a high level of complexity of miRNA target selection and regulation. These deregulated miRNAs highlight new candidate gene targets allowing for a better understanding of the molecular mechanism underlying the development of CML, and propose possible new avenues for therapeutic treatment.
A high positive control (HC) was also provided and included in each run. Each laboratory performed 2 runs with the BCR-ABL IS-MMR kit (Ipsogen) following the kit package insert instructions, and 1 run with their validated in-house method. Eleven laboratories had a HF, and were able to report home-brew results before (HB) or after (HB-CF) conversion to the IS. Four different real-time instruments were used.

**Results.** Technical failure rate with the kit was low: 18 missing measures (7.1%), corresponding to two runs 1 failed in lab 5 and 14. Lab 7 reported a technical problem on its real-time instrument during the kit experiments, but results were not discarded. Inter-laboratory comparison was evaluated on fold-changes, taking the median values observed among laboratories as reference (see figure). On 81 common interpretable measures assessed in 10 laboratories on BCR-ABL positive samples, 85% and 94% of BCR-ABL/ABL% IS obtained with the kit (for run 1 and 2, respectively) were within the 2-fold interval, compared to 74% with HB and only 69% with HB-CF (p=0.00026). Values obtained with the kit were consistent with expected values and HB results, whereas HB-CF median values were systematically below the BCR-ABL/ABL% IS median values obtained with the kit. Kit results were consistent between runs, with 97% overall agreement on MMR prediction. **Conclusions.** In this study, the IS-MMR kit significantly improved inter-laboratory variability compared to HB-CF. With HB methods, laboratory-specific CF did not reduce inter-laboratory variability and even led to an underestimation of BCR-ABL values. Routine practice use of secondary reference materials calibrated on the WHO CRM should therefore be a simple and effective approach to standardize BCR-ABL quantification.
rectly in 71/72 (99%) tests. For the 17 mutated samples, 240/485 (59%) samples were identified correctly. For further analysis, those samples with high (≥20%; n=11) and low (<10%; n=6) levels of mutation were distinguished. For the high level samples 224/264 (85%) tests were reported correctly. Of the incorrect results, 28 were scored as negative and 12 were false positives. For the low level samples, 164/111 (15%) tests correctly identified the mutation. Most (127/144; 88%) were reported as undetectable and a false positive was reported in one case. We conclude that Sanger sequencing is the most frequently applied technique for routine analysis of BCR-ABL1 KD mutations in CML in Europe. In general it reliably identifies mutations when the proportion of mutant alleles comprise 20% or more. Nevertheless, false negative and positive results were reported in a substantial proportion of samples with ≥20% mutation level (40/264, 15%). For mutations that are present at 10% or less mutant alleles, routine methods mainly failed to identify mutations. This study indicates that further work is needed to establish reliable tests to identify BCR-ABL1 KD mutations in CML on TKI therapy.

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NILOTINIB 400 MG BID FRONTLINE: WITH A FOLLOW-UP OF 3 YEARS, RESULTS REMAIN EXCELLENT AND STABLE. (A GIMEMA CML WP 2 PHASE 2 TRIAL)


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Background.Nilotinib is a potent and selective BCR-ABL inhibitor. The phase 3 NEशESTud trial demonstrated superior efficacy of nilotinib vs imatinib, with higher and faster molecular responses. After a median of 24 months, the rates of progression to accelerated-phase disease (APD) were 0.7% and 1.1% with nilotinib 300mg and 400mg BID as frontline therapy, respectively, which was significantly lower compared to imatinib (4.2%). Nilotinib has been approved for the frontline treatment of Ph+ CML. With imatinib 400mg (IRIS trial), the rate of any event and of progression to APD were higher during the first 3 years. Consequently, a confirmation of the durability of responses to nilotinib beyond 5 years is extremely important. Aims: To evaluate the response and the outcome of patients treated for 3 years with nilotinib 400mg BID as frontline therapy. Methods:A multicentre phase 2 trial was conducted by the GIMEMA CML WP (ClinicalTrials.gov NCT00481052). Minimum 36-month follow-up data for all patients will be presented. Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio <0.1%; Complete Molecular Response (CMR): undetectable transcript levels with ≥10,000 ABL transcripts; failures: according to the revised ELN recommendations: events: failures and treatment discontinuation for any reason. All the analysis has been made according to the intention-to-treat principle. Results.73 patients enrolled: median age 51 years; 45% low, 25% intermediate, and 14% high Sokal scores. The cumulative incidence of CCgR at 12 months was 100%. CCgR at each milestone: 82%, respectively. The overall estimated probability of CMR was 79% at 30 months, while the rates of MMR at 12 and 24 months were 12% and 27%, respectively. No patient achieving a MMR progressed to APB. Over 3 years of observation, only one patient progressed to APB, at 6 months, and subsequently died (high Sokal risk, T315I mutation). AEs were mostly grade 1 or 2 and manageable with appropriate dose adaptations. During the first 12 months, the mean daily dose was 600-800mg in 74% of patients. Nilotinib last daily dose was as follows: 800mg in 46 (63%) patients, 600mg in 3 (4%) patients and 400mg in 18 (25%). Six permanent discontinuations: 2/6 deaths (1 ABP, 1 mental deterioration and starvation, unrelated to study drug). 3/6, recurrent episodes of anemia and/or lipase increase (no pancreatitis) and 1/6, atrial fibrillation (other episodes before nilotinib). 2 patients are currently on imatinib second-line and 2 on dasatinib third-line. With a median follow-up of 39 months, the estimated probability of overall survival, progression-free survival and failure-free survival was 97%, the estimated probability of event-free survival was 91%. Conclusions. The rate of failures was very low during the first 3 years. Responses remain stable. The high rates of responses achieved during the first 12 months are being translated into optimal outcome for most of patients.

Acknowledgements: European LeukemiaNet, COFIN, Bologna University, Bologna/AL.

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LONG-TERM EFFICACY AND SAFETY OF DASATINIB 100 MG ONCE-DAILY (QD) IN PATIENTS WITH IMATINIB-RESISTANT/INTOLERANT CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CML-CP): 5-YEAR FOLLOW-UP FROM CA180-034

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Background. For almost 10 years, the BCR-ABL inhibitor imatinib was the standard first-line treatment for CML-CP. Dasatinib, a more potent BCR-ABL inhibitor, is an approved therapy for patients with imatinib-resistant or imatinib-intolerant CML or newly diagnosed CML-CP. The recommended dosing regimen is dasatinib 100 mg once daily (QD), based on results from the CA180-034 dose-optimization study in patients with CML-CP and resistance or intolerance to prior imatinib therapy. The CA180-034 trial provides the longest follow-up of patients with CML-CP treated with a newer BCR-ABL inhibitor. Aims: To investigate the long-term efficacy and safety/tolerability of dasatinib with QD or twice-daily (BID) dosing in patients with CML-CP with resistance or intolerance to prior imatinib therapy. Methods: Details of the study design and endpoints have been published (Shah et al. J Clin Oncol, 2008; 26: 3204-12). Patients (n=670) provided informed consent and were randomized using a 2x2 factorial design to one of four dasatinib dosing regimens: 100 mg QD, 50 mg twice-daily (BID), 140 mg QD, or 70 mg BID. Results. After a minimum follow-up of 4 years, 251 (37%) patients remained on dasatinib treatment. More patients in the dasatinib 100 mg QD arm (45%) remained on treatment compared with the other three arms (33-37%). For patients randomized to dasatinib 100 mg QD (n=167), the 4-year rate of progression free survival (PFS) was 66%, overall survival (OS) was 22%, and 4% of patients had transformed to accelerated or blast phase while on treatment. In the dasatinib 100 mg QD arm, 4-year PFS rates were 93% and 87% in patients who had achieved a complete cytogenetic response (with or without a major molecular response) at 6 and 12 months, respectively. For dasatinib 100 mg QD, 4-year cumulative rates of nonhematologic adverse events (AEs) of any grade included headache (33%), diarrhea (28%), fatigue (25%), and pleural effusion (24%). Most nonhematologic AEs first occurred within 24 months of starting treatment. Grade 3/4 hematologic AEs usually first occurred within the first 12 months of treatment and included neutropenia (46%) and thrombocytopenia (24%). Dose modifications or changes to the dosing schedule were permitted for managing AEs. At the last available follow-up across the four treatment arms, 178 (71%) patients were on a QD dosing schedule, of which 107 (60%) patients were taking ≥100 mg QD. 44% of patients switched
from BID to QD dosing. Five-year follow-up will be presented. Conclu-
sions: In the CA180-034 study, the majority of patients who remain on
study are on a QD dosing schedule after 4 years of dasatinib treatment.
Continued follow-up of patients with CML-CP receiving dasatinib fol-
lowing prior imatinib therapy further demonstrates the durable effi-
cacy and acceptable tolerability and safety profile of long-term dasa-
tinib 100 mg QD treatment.

CML PATIENTS FAILING TO ACHIEVE MMR BY 12 MONTHS MAY
BENEFIT FROM DOSE ESCALATION OR SWITCHING TO NILOTINIB:
A 24 MONTH UPDATE OF RESULTS FROM THE TIDEL-II TRIAL

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Background. We have previously demonstrated excellent results from the
TIDEL-II trial, using sequential therapy (imatinib [IM] then nilo-
tinib [NIl]) in de novo chronic phase CML (CML-CP) patients. Here,
we present longer term results (median follow-up of 24 months). Aim.
To optimise molecular outcome and survival in treatment naive CML-
CP patients by selective dose escalation of imatinib and early switching
to nilotinib based on imatinib blood level and early treatment response.
Method. 105 patients entered the first cohort of this open label multi-
centre prospective trial, conducted through the ALLG. Therapy starts
with IM at 600mg/d. Patients with IM trough level <1000mg/mL on day
22 and those failing to achieve molecular goals (BCR-ABL RQ-PCR of
10%, 1% and 0.1% IS at 8, 6 and 12 months respectively) had their IM
dose escalated to 800mg/d. Patients were switched pre-emptively to
nilotinib for a) failure to achieve molecular goals 3 months after dose
escalation; b) loss of response or c) IM intolerance (Grade III/IV or per-
sistent Grade II non-haematological toxicity). Results. The median
follow-up is 24 months (range: 15-29). The confirmed MMR & CMR
rate was 65% and 12% at 12 months respectively (TTT). Two patients
progressed to AP/BC, both had kinase domain mutations (one T315I
amongst others, the other H396P). There were two deaths, one from
disease progression and one following a myocardial infarct. Seventeen
patients withdrew from the study (16%). In total, six patients devel-
oped mutations (6%), two with T315I (5 patients on IM at the time
mutations were detected). All three patients who experienced disease
progression or T315I mutation had PCR >1% at 3 months, one, six and
11 patients dose escalated from IM600mg/d to IM800mg/d for sub-
tropical responses at 3, 6 and 12 months respectively; 5/7 patients who
dose escalated at 3 and 6 months met their goals after 3 months, al-
though two of these patients subsequently failed their 12 month time
point. In all, 11/18 (61%) met treatment goals within 3 months of dose
escalation. Thirty-seven patients failed to achieve confirmed MMR by
12 months, 5 had withdrawn before that time point. Thirty-two re-
mained on trial with a median additional follow up of 12 months, 10
of whom achieved confirmed MMR (31%) subsequently. Of the 22 pa-
tients who did not achieve MMR, seven had withdrawn (1 BC trans-
formation, 1 T315I mutation, 5 for other reasons); the remaining 15 all
have RQ-PCR between 0.1% and 1%. Conclusions. Selective dose esca-
lation of IM for patients who failed to achieve adequate drug levels or
treatment milestones, with pre-emptive switching to nilotinib, is a suc-
cessful therapeutic strategy in treatment naive CML-CP patients, with
excellent 12 month MMR rates. For those who failed to reach MMR at
12 months, even though a majority have sustained RQ-PCR between
0.1-1%, achieving MMR in the subsequent 12 months is difficult. The
TIDEL-II approach has highlighted the problems in achieving a deeper
response in this group of patients and the importance of early disease
treatment.

THE PROGNOSTIC SIGNIFICANCE OF MOLECULAR, CYTOGENETIC
AND HEMATOLOGIC RESPONSE LANDMARKS AFTER 3 MONTHS
OF IMATINIB IN THE UPFRONT TREATMENT OF CHRONIC MYELOID
LEUKEMIA

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Background. As molecular remission after three months of ima-
tinib treatment has been shown to be associated with a favorable out-
come in terms of failure free survival according to European Leukemi-
aNet (ELN) definitions and progression free survival (Hanfstein et al.,
Blood. ASH Annual Meeting Abstracts,116:360). With the availability of
potent second generation tyrosine kinase inhibitors (TKI) for upfront
treatment of CML, the early identification of patients at risk under ima-
tinib is crucial for clinical decision making and patients' outcome.
According to ELN recommendations (Baccarani et al., JCO 2009) patients
are considered treatment failures if they lack a complete hematologic
remission (CHR) after three months of imatinib treatment. If no cyto-
genic response is observed after three months (No CgR, Ph+
metaphases >95%) patients are considered suboptimal responders.
Molecular landmarks offering guidance in the interpretation of three
month BCR-ABL levels are lacking up to now. Aims. We sought to com-
pare the predictive significance of early molecular, cytogenetic and
hematologic landmarks to evaluate new and established response cri-
cera in a large data set. METHODS: In the randomized German CML
Study IV n=949 patients were treated with an imatinib based therapy
consisting of standard dose imatinib (400 mg/d, n=265), high dose ima-
tinib (800 mg/d, n=260) and combinations of standard dose imatinib
with low dose cytarabine (n=138) or interferon alpha (n=236). BCR-
ABL IS was determined by quantitative RT-PCR. The type of BCR-ABL
transcript (b2a2, n=424; b3a2, n=464; b2a2 and b3a2, n=148) was de-
defined by multiplex PCR. Patients with atypical BCR-ABL transcripts
were excluded from the analysis. Cytogenetic response was deter-
mined by G-banding metaphase analyses. Disease progression was de-
defined by the incidence of accelerated phase, blastic phase or death on
an intention-to-treat basis. In total 49 progressions were observed after
a median of 22 months (range 1-70), 37 patients died. 441 patients were
evaluable for hematologic, 423 for cytogenetic and 570 for molecular
prediction after three months. Log-rank tests were used to analyze Ka-
pling and event time with the Kaplan-Meier method. RESULTS: Trea-
tment failure after three months defined by the lack of CHR (n=94)
did not prove to be predictive for disease progression (p=0.5). Suboptimal
response defined as no CgR, Ph+ metaphases >95% showed a trend to
significance (p=0.0553). In contrast, the 10% BCR-ABL IS landmark separated a high risk group (n=161) with a 17% risk
for disease progression after 7 years from a low risk group with a 4%
risk (Kaplan-Meier estimate, p=0.0156). The lack of a partial cyto-
genic remission (Lack of PGCR, Ph+ metaphases >85%) is suited as a
cutoff level as well (p=0.0367). Conclusions. After three months of ima-
tinib treatment high risk patients can be identified by a BCR-ABL of
10% or more, whereas the lack of a complete hematologic remission is
t of no prognostic relevance.
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NILOTINIB EXPOSURE-RESPONSE ANALYSIS IN PATIENTS WITH IMATINIB-RESISTANT OR -INTOLERANT CHRONIC MYELOID LEUKEMIA (CML)
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Background. Nilotinib is approved for the treatment of patients with newly diagnosed Ph+ CML in chronic phase (CML-CP), and Ph+ CML-CP and accelerated phase (AP) resistant or intolerant to imatinib. Annals. To evaluate the population pharmacokinetics (PK) of nilotinib and its relationship with safety and efficacy in patients with imatinib-resistant or -intolerant CML. Methods. Population PK was assessed by non-linear mixed effects modeling, including 495 CML patients in CP (n = 235), AP (n = 135), or blast crisis (BC, n = 125) who had nilotinib PK data available. Exposure-efficacy analysis was performed in CML-CP patients, where the efficacy measures included complete cytogenetic response (CCyR) at 12 and 24 months, major molecular response (MMR) at 12 and 24 months, time to CCyR, time to MMR, and time to progression (TTP; defined as time from study entry to discontinuation due to disease progression or death). Baseline prognostic risk score (0 to 2) was investigated as a covariate in the exposure-efficacy analysis: 0 if hemoglobin > 120 g/L, basophils < 4%, and no insensitive mutation; 1 if missing 1 criterion; 2 if missing 2 or 3 criteria (Kantarjian et al., ASH 2009). The relationship between nilotinib Cmin, UGT genotype, and total bilirubin levels over 24 months was assessed in all patients. Results. Nilotinib dose intensity and PK were similar in all phases of CML. Baseline demographics did not significantly affect nilotinib PK. Patients with lower nilotinib dose and Cmin (Q1) tended to have lower CCyR at 12 months, lower MMR at 12 and 24 months, longer time to CCyR and MMR, and shorter TTP than patients with higher nilotinib dose and Cmin (Q2-Q4, Table). Baseline prognostic risk score also significantly affected TTP (2.79 years for score ≤1 vs 2.25 years for score 3 ≥2). Nilotinib Cmin and UGT genotype were significantly associated with the occurrence of bilirubin abnormalities (both P < .1). The occurrence of grade 3/4 bilirubin abnormalities was 6%, 10%, 10%, and 14% in patients with nilotinib Cmin in Q1 (≤429 ng/mL, n = 124), Q2 (429-<615 ng/mL, n = 123), Q3 (615-<850 ng/mL, n = 123), and Q4 (≥850 ng/mL, n = 123), respectively, and 6%, 12%, and 48% for patients with TA(6)/TA(6), TA(6)/TA(7), and TA(7)/TA(7) UGT genotypes, respectively. Conclusions. Patients with lower nilotinib dose, lower Cmin, and higher baseline prognostic risk showed a higher risk of progression and a trend of poorer response. Nilotinib Cmin was also significantly associated with total bilirubin elevation. However, the observed hyperbilirubinemia was clinically manageable in patients receiving nilotinib therapy. The present analysis suggests that adherence to nilotinib dose in order to maintain sufficient Cmin is important in maximizing the clinical efficacy of nilotinib.

Table 1.

<table>
<thead>
<tr>
<th>Cmin Quartile</th>
<th>CCyR at 24 months</th>
<th>MMR at 24 months</th>
<th>TTP (months)</th>
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<tr>
<td>Q1</td>
<td>86%</td>
<td>70%</td>
<td>1.9</td>
</tr>
<tr>
<td>Q2-Q4</td>
<td>90%</td>
<td>82%</td>
<td>2.0</td>
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0140
LYMPHOCYTE MOBILIZATION AFTER DASATINIB INTAKE IS CORRELATED WITH PLASMATIC LEVEL OF DASATINIB AND MAY INFLUENCE MOLECULAR RESPONSE IN CP-CML PATIENTS RECEIVING DASATINIB AS SECOND LINE THERAPY
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Background. Lymphocytes mobilization after dasatinib intake has been recently reported (S Mustjoki et al., ASH Annual Meeting Abstracts; 116: 1204). The clinical significance of this phenomenon is not clearly established. We aimed to describe lymphocytes mobilization in Chronic Phase Chronic Myelogenous Leukemia (CP-CML) patients receiving dasatinib as a second line therapy. Patients and Methods. CP-CML patients from two institutions and receiving dasatinib were prospectively proposed to perform a WBC and a platelets analysis before (H0) and 2 hours (H2) after dasatinib intake. Dasatinib plasmatic levels were determined on the same time points (Cmin and C2hours). BCR-ABL/ABL IS ratio was determined during the same period of time. Results. 40 CP-CML patients were analysed and we report here the data on the first 28 patients studied. Median age was 59.6y (27-90) and M/F ratio (M/F) was 1.5. The Sokal risk group was low in 45%, intermediate in 25% and high in 30% of the patients. 48% of the patients received dasatinib after imatinib failure and 52% after imatinib intolerance. The median duration of dasatinib before the lymphocytes analysis was 29 months (0.4-68). The median lymphocytes count values at H0 was 1.27 G/L and 2.6 G/L at H2 (paired t-test p<0.001). Surprisingly, a transient decrease in the platelets counts was observed between H0 and H2 (195.5 G/L versus 167.5 G/L, paired t-test p<0.001). This was associated with a transient increased of the occlusion time. The magnitude of lymphocytes mobilization (H2/H0) was 1.82 fold in median (1-3.56). We next analyzed if the lymphocytes increment was linked to pharmacokinetic (PK) values. No correlation was found with the Cmin. However, a positive correlation was demonstrated with the 2 hours plasmatic level of dasatinib (r²=0.5736, p=0.0011). We then compared the magnitude of lymphocytes mobilization in patients with or without major molecular response (MMR). A significant association was found between high levels of mobilization and MMR (p=0.047). A phenotypic analysis was conducted in 7 patients. Despite a pan lymphocytes mobilization, a preferential increase in the CD8~/CD16/56~ and in the CD3~/CD57+ lymphocytes populations was observed (1.83 and 2.94 fold in median respectively). Of note, large granular lymphocytes was reported in only 2 patients. No patient had an absolute lymphocytosis. We also confirm that lymphocytes mobilization could be observed in virtually all patients receiving dasatinib in the absence of an absolute lymphocytosis. We also confirm that lymphocytes mobilization is driven by dasatinib PK and report for the first time a link between lymphocytes mobilization and MMR. More patients will be presented and we are currently analysing lymphocytes mobilization in our first line patients included in the OPTIM dasatinib French trial.
MOLECULAR RESPONSE <1% BCR-ABL (IS) AT 12 MONTHS IS ASSOCIATED WITH IMPROVED OVERALL AND PROGRESSION-FREE SURVIVAL AND REPRESENTS A SUPERIOR PREDICTOR THAN COMPLETE CYTOGENETIC REMISSION

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Background. The prognostic relevance of major molecular remission (MMR, ≤0.1% BCR-ABL according International Scale, IS) for survival on imatinib-based treatment has remained uncertain. Gold standard is complete cytogenetic remission in spite of its limited sensitivity, the need for bone marrow. Standardization of PCR, definition of a uniform reporting system, and harmonization of laboratories using conversion factors to account for differences of methods and reagents have changed the situation in Europe. Aims. To determine the degree of molecular response after 12 months of imatinib-based treatment and its impact on overall and progression-free (PFS) survival. Methods. 648 out of 1014 patients within the CML-Study IV (randomized comparison of imatinib 800 mg vs 400 mg + IFN) were eligible for evaluation since 12 month molecular data was available. Patients (61% male) have been recruited between July 2002 and April 2009, the median age was 54 years (range 16-84), and the median observation time was 40 months (minimum 12). Landmark analyses at 12 months and log-rank tests were performed. Results. 541 patients (40%) achieved a BCR-ABL expression ≤0.1% (MMR), 240 patients (28%) between 0.1% and 1% and 267 patients (31%) >1% by 12 months. Independent of treatment approach, the groups of patients achieving MMR and 0.1%<1% at 12 months showed significantly higher PFS (97% vs 95% vs 87% at 5 years, p=0.0023), and better overall survival (97% vs 96% vs 88% at 5 years, p=0.001) compared to the group with >1% BCR-ABL by 12 months. Applying a landmark analysis at 12 months depending on the achievement of complete cytogenetic remission (CCyR) revealed less pronounced differences in overall survival (96% vs 91% at 5 years, p=0.015) than using the molecular predictor. Conclusions. Faster and deeper response to imatinib-based treatment by 12 months seemed to be associated with improved PFS and overall survival. The critical cut-off level seems to be 1% BCR-ABL IS which is supposed to closely correlate with complete cytogenetic remission. Furthermore, RQ-PCR from peripheral blood is more precise and better tolerable than cytogenetics from bone marrow.

BOSUTINIB AS THIRD-LINE THERAPY FOR CHRONIC PHASE CHRONIC MYELOID LEUKEMIA FOLLOWING FAILURE WITH IMATINIB AND DASATINIB OR NILOTINIB

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Background. Bosutinib is an orally active, dual Src/Abl tyrosine kinase inhibitor (TKI) with minimal inhibitory activity against PDGFR or c-kit. Aim. This open-label, phase 1/2 trial evaluated the safety and efficacy of bosutinib as third-line therapy in patients with Philadelphia chromosome-positive chronic phase chronic myeloid leukemia (CP CML). Methods. After informed consent was obtained, adults (aged ≥18 years) with prior imatinib failure who were dasatinib-resistant (n = 57), dasatinib-intolerant (n = 50), or nilotinib-resistant (n = 27) received daily bosutinib (median dose 562 mg). Results. 141 patients were enrolled, 126 of whom were evaluable (32% male). Median time of CML diagnosis was 6.3 years (range, 0.6-18.3 years). Median follow-up was 26.4 months (range, 0.3-54.0 months), and the median bosutinib dose was 476 mg/day (range, 185-563 mg/day). Non-hematologic treatment-emergent adverse events (TEAEs) seen in ≥20% of patients (all grades/grade ≥3) included diarrhea (95%/9%), nausea (46%/1%), vomiting (40%/0%), rash (27%/4%), headache (25%/3%), fatigue (21%/1%), and abdominal pain (20%/1%). The incidence of TEAEs was similar for dasatinib-resistant, dasatinib-intolerant, and nilotinib-resistant patients. Gastrointestinal events were predominantly grade
LONG TERM OUTCOME OF CHRONIC MYELOID LEUKEMIA ELDERLY PATIENTS TREATED FRONTLINE WITH IMATINIB. A SURVEY BY THE GIMEMA CML WP


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Background. The median age of Chronic Myeloid Leukemia (CML) patients is around 60 years and age is still considered an important prognostic factor, included in Sokal and EUO risk scores. With IM, this negative impact of age has been partially reappraised, at least in late chronic phase (CP). However, only limited data about the long term outcome are available for elderly patients treated in early CP, moreover, the allocation of elderly patients to IM, in particular within clinical trials, is still an important issue. Aim. To evaluate the long term outcome of elderly patients treated frontline with IM. Methods. We analyzed the relationship between age and outcome in 539 early CP CML patients, enrolled in 3 prospective clinical trials of GIMEMA CML-WP (Clin. Trials Gov. NCT00514488 and NCT00510926), treated with IM. The median follow-up was 60 months (range 1-83 months). Older patients (≥ 65 years old) were 115 (21%). Events were defined as: treatment failure or permanent discontinuation of IM for any reason; treatment failures were defined according to the updated European LeukemiaNet (ELN) recommendations; progression to accelerated/blast phase was defined according to ELN criteria. Results. The hematologic, cytogenetic, and molecular response rates observed in the 2 age groups were similar. The cumulative incidence of complete cytogenetic response (CCyR) and major molecular response (MMR) were 87% (100/115) and 85% (99/115) vs. 85% (591/444) and 85% (377/444), in older and younger patients, respectively. CCyR at 6, 12, and 18 months were 79/115 (69%), 90/115 (78%), and 85/115 (75%) in older patients, respectively, and 299/444 (67%), 345/444 (77%), and 346/444 (78%) in younger ones, respectively; MMR at 6, 12, and 18 months were 54/115 (47%), 67/115 (58%), and 65/115 (57%) in older patients, respectively, and 262/444 (45%), 275/444 (52%), and 278/444 (63%) in younger ones, respectively; all the differences were not significant. Regarding the long term outcome, the estimated 6-year EFS (55% vs. 60%, p = 0.006), FFS (62% vs. 78%, p = 0.009), DFS (75% vs. 90%, p = 0.0001) and OS (78% vs. 92%, p = 0.0001) were all significantly worse in the older age group. Importantly, the analysis of the causes of death showed that in older patients more deaths in complete hematologic response (CHR) (unrelated to CML progression) have been recorded: 17/115 (15%) and 15/444 (3%) for older and younger patients, respectively (p = 0.001). On the other hand, deaths due to progression of CML were 115/6 (15%) and 15/444 (0%), for older and younger patients, respectively (p = 0.4). The analysis of the estimated 6-year EFS, DFS, OS, and MMR at 18 months were 79/115 (69%), 90/115 (78%), and 85/115 (75%) in older patients, respectively, and 299/444 (67%), 345/444 (77%), and 346/444 (78%) in younger ones, respectively; all the differences were not significant. Regarding the long term outcome, the estimated 6-year EFS (55% vs. 60%, p = 0.006), FFS (62% vs. 78%, p = 0.009), DFS (75% vs. 90%, p = 0.0001) and OS (78% vs. 92%, p = 0.0001) were all significantly worse in the older age group. Importantly, the analysis of the causes of death showed that in older patients more deaths in complete hematologic response (CHR) (unrelated to CML progression) have been recorded: 17/115 (15%) and 15/444 (3%) for older and younger patients, respectively (p = 0.001). On the other hand, deaths due to progression of CML were 115/6 (15%) and 15/444 (0%), for older and younger patients, respectively (p = 0.4). The analysis of the estimated 6-year EFS, DFS, OS, and MMR at 18 months were 79/115 (69%), 90/115 (78%), and 85/115 (75%) in older patients, respectively, and 299/444 (67%), 345/444 (77%), and 346/444 (78%) in younger ones, respectively; all the differences were not significant.
tion to 400 mg BID for SoR or TF after a median of 14 months of treatment with imatinib 400 mg QD. Median time on treatment after dose escalation among these patients was 9 months (range 0.2 – 28.2). Subsequent to dose escalation, 21 (26%) patients achieved MMR; 14 (17%) patients achieved CCyR without MMR, and 47 (57%) patients had no improvement in responses after dose escalation. After dose escalation, 36 (44%) patients had dose reductions / interruptions. A total of 37 (45%) patients discontinued treatment subsequent to imatinib dose escalation: 23 (28%) due to SoR or TF; 6 (7%) due to intolerance, 3 (4%) due to disease progression, and 5 (6%) due to other reasons. Conclusions. Frontline therapy with nilotinib offers improvement over the standard of care imatinib 400 mg QD in terms of less frequent SoR and TF. Favorable long-term outcomes are unlikely in patients with TF, and may be less likely in patients with SoR than in optimal responders. Dose escalation was not an effective strategy to overcome SoR/TF on imatinib, as approximately three quarters of these patients failed to achieve MMR after dose escalation, and nearly half had dose reductions/interruptions and discontinued treatment. Using 2009 ELN criteria, nilotinib frontline therapy (especially 300 mg BID) appears to be more effective than imatinib frontline therapy, even with imatinib optimization by dose escalation.

Table 1.

0146

BOSUTINIB (SKI-606) AS SECOND-LINE THERAPY FOR CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CP CML) FOLLOWING IMATINIB FAILURE: ANALYSES OF CROSS-INTOLERANCE AND RESPONSE BY PRIOR RESPONSE TO IMATINIB

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Background. Bosutinib is an orally active, dual Src/Abl tyrosine kinase inhibitor (TKI) with minimal inhibitory activity against FGFR or c-kit. Aims. This open-label, phase 1/2 study evaluated the efficacy and safety of bosutinib as second-line therapy in patients with Philadelphia chromosome-positive CP CML. Methods. After collection of informed consent, adults (aged ≥18 years) with resistance (n = 200) or intolerance (n = 88) to imatinib received daily oral bosutinib starting from 400 to 600 mg/day. Results. Of 288 CP patients enrolled, 55% were male, median age was 58 years (range, 18-91 years), and median time from CML diagnosis was 3.6 years (range, 0.1-15.1 years). Median follow-up was 2.5 years (range, 0.1-5.0 years), with 49% of imatinib-resistant and 47% of imatinib-intolerant patients still receiving treatment. Median bosutinib dose was 485 mg/day (range, 128-600 mg/day). Of 172 (66%) imatinib-resistant and 75 (35%) imatinib-intolerant patients achieving complete hematologic response (CHR), respectively, 69% and 81% retained their response as of the data cutoff. In the evaluable population, of 101 (54%) imatinib-resistant and 39 (49%) imatinib-intolerant patients achieving major cytogenetic response (MCyR), respectively, 66% and 90% retained their response as of the data cutoff. Of patients who had not previously achieved MCR on imatinib, 45% and 28% achieved MCyR and CCyR, respectively, on bosutinib (Table). Complete cytogenetic response (CCyR) was achieved by 42% and 45% of imatinib-resistant and imatinib-intolerant patients, respectively. Among patients who achieved CCyR, major molecular and complete molecular (undetectable Bcr-Abl transcripts) responses, respectively, were observed in 69% and 52% of evaluable imatinib-resistant and 73% and 68% of evaluable imatinib-intolerant patients. MCyR and CHR were observed across 25 different Bcr-Abl kinase domain mutations, but not T315I. Grade ≥3 non-hematologic treatment-emergent adverse events (TEAEs) seen in ≥2% of patients were diarrhea (9%), rash (9%), and vomiting (5%). Diarrhea was predominantly grade 1/2, early in onset, and usually subsided within a month. Grade 3/4 lab abnormalities (≥10%) included thrombocytopenia (24%), neutropenia (17%), anemia (13%), hypomagnesemia (11%), and alanine transaminase elevation (10%). Overall, dose reductions and interruptions were required by 47% and 67% of patients, respectively; 12% of patients dose escalated to bosutinib 600 mg/day. TEAEs led to treatment discontinuation in 17% of imatinib-resistant and 53% of imatinib-intolerant patients. Limited cross-intolerance between bosutinib and imatinib was observed: 8% of patients with imatinib intolerance re-experienced the same grade ≥3 AE on bosutinib and 11% discontinued bosutinib due to the same AE. Four of 32 patients with imatinib intolerance related to myelosuppression experienced grade ≥3 myelosuppression on bosutinib, and 3 of 10 patients with imatinib intolerance related to rash experienced a grade ≥3 rash on bosutinib; no patient with imatinib intolerance related to gastrointestinal events, edema, fatigue, hepatobiliary disorders, or muscle spasms experienced these grade ≥3 events on bosutinib. Summary/Conclusions. Bosutinib demonstrated promising activity as second-line therapy, with responses observed irrespective of prior response to imatinib. Bosutinib was also associated with an acceptable toxicity profile, with limited cross-intolerance. These results emphasize the therapeutic potential of bosutinib in patients with CP CML following imatinib failure.

Table 1.

0147

IMPACT OF CYTOGENETICS AT DIAGNOSIS ON OUTCOME OF CML: RESULTS FROM THE RANDOMIZED GERMAN CML STUDY IV

A Fabarius,1 A Leitner,1 A Hochhaus,1 C Haferlach,2 G Goehring,1 B Schlegelberger,1 A Reiter,1 S Jung-Munkwitz,1 U Proetel,1 M Lauseker,1 M Pfirrmann,1 J Hasford,1 WK Hofmann,1 S Saussele,1 R Hehlmann1

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Background. The prognostic impact of cytogenetic findings at diagnosis to the translocation t(9;22)(q34;q11) or the variant translocation haematologica | 2011; 96(s2) | 59
t(v;22) at diagnosis of chronic myeloid leukemia (CML) is conflicting. Patients and Methods. We used baseline and outcome data of 1030 patients (pts) (808 male, 422 female, median age 53, range 16-88) with chronic phase CML randomized to the German CML-Study IV (imatinib [IM] 800 mg [n=265] vs IM 400 mg [n=254] vs IM 400 mg + IFN [n=281] vs IM 400 mg after IFN failure [n=108] vs IM 400 mg + AraC [n=122]). We sought to investigate the impact of additional clonal cytogenetic findings on time to complete cytogenetic remission (CCR), as an accepted prognostic marker. Cytogenetic analysis was performed after 24- and/or 48 h culture on G-banded metaphases. If appropriate, fluorescent-in-situ-hybridization (FISH) was used in addition. Results. In total, at diagnosis 912/1030 pts (89%) had the translocation t(9;22)(q34;q11), 60/1030 pts (5.9%) showed a variant translocation t(v;22), 58/1030 (5.6%) had additional clonal cytogenetic findings [56 pts in addition to the translocation t(9;22) and 2 pts in addition to a variant translocation t(v;22)]. Out of these 10/5030 pts (2.9%) lacked the Y chromosome (-Y) and 28/1030 pts (2.7%) had additional numerical or structural aberrations except -Y. Median age, sex and treatment were similarly distributed except for -Y male pts who were older. For pts with the translocation t(9;22), with variant translocations t(v;22) and with the translocation t(v;22)(q34;q11)/t(v;22) with additional chromosomal changes other than -Y, median time (years) to CCR was 0.98, 0.84 and 1.92. When comparing the groups *translocation t(9;22) and t(v;22) with additional chromosomal findings other than -Y* and when comparing the groups *variant translocations t(v;22)* and *translocation t(v;22)/t(v;22) with additional chromosomal findings other than -Y* over the entire period of time, time to CCR was significantly shorter in the group *translocation t(9;22) respectively *variant translocation t(9;22)* (p=0.001). No difference regarding time to CCR occurred when comparing the groups *translocation t(9;22)* and *t(v;22)* or the groups *translocation t(9;22) and -Y*. Conclusions. We conclude that additional clonal cytogenetic findings at diagnosis have an impact on time to CCR and possibly on prognosis.

0148
FISH PATTERNS AND TREATMENT OUTCOME IN 12 PH-NEGATIVE CML CASES
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Sverdlovsk Regional Hospital I, Ekaterinburg, Russian Federation
Clinical Medical Sanitary Unit 1, Perm, Russian Federation
Kurgan Regional Hospital I, Kurgan, Russian Federation
BU Oncology, Novartis Pharma, Ekaterinburg, Russian Federation
Ural State Medical Academy, Ekaterinburg, Russian Federation

Background. Approximately 1% of the CML patients do not have t(9;22)(q34;q11) detectable by conventional cytogenetics, but carry BCR-ABL fusion gene revealed by fluorescence in situ hybridization (FISH) and/or reverse-transcriptase PCR (RT-PCR). Imatinib efficacy in this group of patients remains unclear. Aim. To define FISH patterns and imatinib treatment efficacy in Ph-negative BCR-ABL-positive CML patients. Methods. Initial diagnostics including chromosome banding analysis (CBA) and RT-PCR was done in 551 primary CML patients. CBA was performed after 24 hours culture. G-bandng was performed by a trypsin-Giemsa method. Karyotypes were described according to ISCN (2005). At least 20 metaphases for each sample were analyzed. In vivo FISH was performed after 24- and/or 48 h culture on G-banded metaphases. Dual-Colour Dual-Fusion BCR-ABL Translocation Probe (Abbott, USA) was used in at least 200 interphase nuclei (I-FISH) and on all available metaphases. CBA and FISH were performed at the time of diagnosis and every 3-6 months of imatinib treatment. Quantitative measurement of BCR-ABL/I-FISH transscripts ratio by real-time quantitative PCR (RQ-PCR) was done every 3-6 months. Detection of point mutations in the BCR-ABL tyrosine kinase domain was performed by direct sequencing of RT-PCR products. Compete cytogenetics response (CCG) was assumed as ≥ 1 % of Ph-positive nuclei (N. Testoni, Blood, 2009) Even when stable response (ESR) was calculated in respect of induction-to-treat and defined as the time from imatinib beginning until any of the following events occurred: any sign of treatment failure (according to the European LeukemiaNet criteria (M. Baccarani et al., JCO, 2009), progression to AP/BC or death of any reason. Results. Normal karyotype was detected in 12 newly diagnosed CML patients (2.3%). However, all of them harboured BCR-ABL fusion gene revealed by FISH and RT-PCR. Ph-negative group included 2 males and 10 females with median age of 51 years and median follow-up of 39-months. 4 patients had e13a2 transcript variant, 8 patients - e14a2. Three different FISH patterns were indentified (table).

Table 1.

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>FISH patterns</th>
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| 7 of 12 patients received imatinib treatment. 6 of 7 patients achieved complete hematological response at 3 months. Median number of BCR-ABL-positive nuclei at 12 months was 22% (range 1-50%), at 18 months - 40% (range 0-92%). Only 1 patient had CCGR (assessed by I-FISH) by 18 months. One patient progressed to AP and died subsequently. None of patients had BCR-ABL mutations. EFS in Ph-negative CML patients treated by imatinib was significantly lower than in 244 Ph-positive ones, for whom long-term follow-up data was available: 0.14±0.13 vs 0.62±0.08 (p=0.007), while overall survival was comparable in both groups: 0.83±0.15 vs 0.85±0.02% (p=0.88). Conclusions. Our data suggests that main mechanisms of fusion gene formation in Ph-negative CML are cryptic insertions of ABL into BCR, or vice versa. In our series treatment outcome in this group was significantly worse in comparison with Ph-positive CML patients. Resistance in observed group seems to have BCR-ABL-independent mechanisms, because of lack of BCR-ABL mutations, duplication or amplification. Absence of response criteria for data obtained by I-FISH assay hampered to refer such patients to treatment failure earlier than 18 months.

0149
CHRONIC PHASE CML PATIENTS ON TYROSINE KINASE INHIBITORS HAVE A POLYFUNCTIONAL CELLULAR IMMUNE RESPONSE AGAINST INFLUENZA BUT AN IMPAIRED IGM HUMORAL RESPONSE AGAINST PNEUMOCOCCUS AFTER VACCINATION
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The tyrosine kinase inhibitors (TKI) imatinib, nilotinib and dasatinib are remarkably effective as single-agent treatments for chronic myeloid leukaemia (CML) in chronic phase (CP). However little is known of their potential impact on the immune system; this is of particular interest in terms of the long-term effects of TKI on tumour immune surveillance and susceptibility to infections. To date there are no human in vivo studies to address how these molecular-targeted drugs affect immune function in patients and data from in vitro and animal studies with imatinib have been contradictory, ranging from impaired antigen-specific T-cell response to enhanced stimulation of tolerant T cells. Few data are available to assess potential immunomodulatory effects of the second-generation TKIs nilotinib and dasatinib. The aim of this study was to prospectively analyze humoral and cellular immune responses to vaccination against influenza virus (Flu) and Pneumococcus in CP-CML patients treated with imatinib, dasatinib or nilotinib compared to healthy controls. Fifty CP-CML patients on standard dose TKIs (IM, n=22; dasatinib, n=15; nilotinib, n=15) and 15 healthy controls were vaccinated against Flu (Influenza vaccine Ph. Eur. 2008/2009, CSL biotherapies) and Pneumococcus (Pneumovax II, Sanofi Pasteur MSD). Samples were collected before and 1 and 3 months post-vaccination. We analyzed the immunological T-cell response to influenza virus both quantitatively and qualitatively using flow cytometry for intracellular TNF-α, IFN-γ, IL-2 and the cytotoxicity marker CD107a. Titers of IgM and IgG anti-pneumococcal were determined using ELISA, and the proportion of B-
cell subsets (IgM memory and switched memory B-cells) were measured using flow cytometry. CD8 and CD4 T-cell responses to Flu vaccination were not significantly different between patients and controls: after vaccination, T-cell responses against Flu were detected in 18/35 patients and 18/35 healthy controls. Polyfunctional T-cell responses against Flu were not significantly different when comparing patients on imatinib, dasatinib and nilotinib although the relatively small number of patients in each group precludes any definitive conclusions. These data have significant implications for the use of TKI in vivo. However, we found that compared to controls, patients on TKI have an impaired IgM antibody response to pneumococcal, associated with a selective reduction in the IgM memory B cell subset. We are currently investigating the mechanism underlying this observation.

Aims.

We aimed to evaluate the efficacy and safety of higher doses of imatinib (≥600 mg daily) compared with standard doses (400 mg daily) for newly diagnosed, previously untreated CP-CML patients. Methods. Systematic review and meta-analysis of randomized controlled trials comparing frontline treatment with single agent imatinib 400 mg daily vs. higher doses (≥600 mg daily) in patients with CP-CML. The Cochrane Library, MEDLINE, conference proceedings and references were searched until February 2011. Two reviewers appraised the quality of trials and extracted data. Outcomes assessed were: Complete cytogenetic response (CCyR) and major molecular response (MMoR) at 12 months; progression to accelerated phase (AP) / blastic crisis (BC); all-cause mortality at the end of follow-up. Adverse events re- sulting discontinuation were more common in the high dose imatinib arm (RR 1.15, 95% CI 1.06-1.24, 4 trials, figure). High dose imatinib also improved MMoR at 12 months (RR 1.36, 95% CI 1.08-1.70, 4 trials). However, there was no difference in all-cause mortality (RR 0.79, 95% CI 0.58-1.08, 4 trials), RR 1.91, 95% CI 1.28-2.93 (3 trials) and RR 2.44, 95% CI 1.21-4.92 (2 trials), respectively. There was no difference in grade III/IV anaemia or myelalgias. Conclusions. Higher doses of imatinib significantly improved CCyR and MMoR, as compared to standard dose imatinib. There was no difference in all-cause mortality or progression to AC/BC, although this should be taken with reservation due to the short time of follow-up. More frequent higher doses were associated with higher toxicity.

Table 1. Complete cytogenetic response at 12 months.

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<tr>
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For the treatment of newly diagnosed, previously untreated chronic myeloid leukemia patients in chronic phase - systematic review and meta-analysis.

A Gafter-Givili,1 A Leader,1 R Gurson,1 I Vidal,1 R Ram,1 A Shatin-Abulafia,1 Ben-Bassat,1 M Lishner,1 O Shlipberg,1 P Raanan2

1 Rabin Medical Center, Petah-Tikva, Israel
2Meir Medical Center, Kfar-Saba, Israel

Background. Imatinib at a dose of 400 mg daily is considered frontline treatment in chronic phase (CP) chronic myeloid leukemia (CML). Doses of 600 mg or 800 mg daily have proven efficacy in the accelerated phase or in the event of unsatisfactory response to standard dose imatinib. Recent randomized controlled trials of standard dose versus higher doses of imatinib as first-line treatment for CP-CML have demonstrated conflicting results regarding treatment outcomes. Aims. We aimed to evaluate the efficacy and safety of higher doses of imatinib (≥600 mg daily) compared with standard doses (400 mg daily) for newly diagnosed, previously untreated CP-CML patients. Methods. Systematic review and meta-analysis of randomized controlled trials comparing frontline treatment with single agent imatinib 400 mg daily vs. higher doses (≥600 mg daily) in patients with CP-CML. The Cochrane Library, MEDLINE, conference proceedings and references were searched until February 2011. Two reviewers appraised the quality of trials and extracted data. Outcomes assessed were: Complete cytogenetic response (CCyR) and major molecular response (MMoR) at 12 months; progression to accelerated phase (AP) / blastic crisis (BC); all-cause mortality at the end of follow-up. Adverse events resulting discontinuation were more common in the high dose imatinib arm (RR 1.15, 95% CI 1.06-1.24, 4 trials, figure). High dose imatinib also improved MMoR at 12 months (RR 1.36, 95% CI 1.08-1.70, 4 trials). However, there was no difference in all-cause mortality (RR 0.79, 95% CI 0.58-1.08, 4 trials), RR 1.91, 95% CI 1.28-2.93 (3 trials) and RR 2.44, 95% CI 1.21-4.92 (2 trials), respectively. There was no difference in grade III/IV anaemia or myelalgias. Conclusions. Higher doses of imatinib significantly improved CCyR and MMoR, as compared to standard dose imatinib. There was no difference in all-cause mortality or progression to AC/BC, although this should be taken with reservation due to the short time of follow-up. More frequent higher doses were associated with higher toxicity.

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A Gafter-Givili,1 A Leader,1 R Gurson,1 I Vidal,1 R Ram,1 A Shatin-Abulafia,1 Ben-Bassat,1 M Lishner,1 O Shlipberg,1 P Raanan2

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0152
THE PREVIOUS RESPONSE TO IMATINIB IS THE MAIN PREDICTOR OF CCYR ACHIEVEMENT ON SECOND LINE TKI TREATMENT

A Zaritskay,1 V Shuvaev,1 A Abdulkadyrova,1 E Usacheva,1 V Udaliev,1 I Zotova,1 I Martinkievitch,1 E Machulaitene,1 N Ilina,1 E Koryagina,1 E Goryunova,1 E Sibitakova,1 N Lazorko,1 E Lomaia,1 K Abdulkadyrov2

1St-Petersburg Pavlov’s State Medical University, St-Petersburg, Russian Federation
2Russian Research Institution of Hematology and Transfusiology, St-Petersburg, Russian Federation

Background. New tyrosine-kinase inhibitors (TKI) is the best possibility to overcome the imatinib resistance in patients with chronic myeloid leukemia (CML). Still we need more data to predict TKI’s efficacy for appropriate choose treatment strategy. Aims. The aim of the study was to evaluate several prognostic markers of new TKI’s efficacy in imatinib resistant patients. Patients and Methods. 47 imatinib-resistant pts (male:female ratio 21:26) with chronic phase of CML were analyzed retrospectively. Patients were treated with nilotinib (34 pts) or dasatinib (13 pts) since 2005 to 2010 with median follow-up on new TKIs 16,4 mos (3-66 mos). The median imatinib pretreatment time was 27 mos (2-83 mos). The median follow-up duration from the diagnosis was 45 mos (8-190 mos). The median age at the beginning of new TKI was 50,4 years (26-84). There were 11, 17, 14 pts with low, intermediate and high Sokal risk, respectively (among 42 evaluable patients). Results. Probability of CCyR achievement was 65 % (the rate 51,1% (23/45)). It was significantly higher among secondary than in primary resistant patients: 81% (rate 66,7% (10/15)) vs 50% (rate 39,3% (11/23)), p=0,045. It also depends on the best cytogenetic response (CCyR, vs >65% Ph-positive cells) during imatinib treatment: 75% (rate 67,9% (19/25)) and 41% (rate 25% (4/16)), p=0,02. The probability of CCyR was higher in pts with complete hematologic response (CHR) at the start of 2nd TKI treatment: 78% (rate 66,7% (20/30)) with CHR vs 18% (rate 2/14) without CHR, p=0,01. It also was statistically but not significantly higher in low-intermediate than in high Sokal risk groups: 80% (rate 65% (17/27)) vs 50% (rate 38,5% (5/15)), respectively, p=0,10. In multivariant analysis (Cox regression) the significance of CHR and best CyR were confirmed, p<0,05. Conclusion. The previous efficacy of imatinib and stable CHR at the start of therapy, but not Sokal risks, appeared the level of stability in patients on imatinib therapy. Methods. Fifty three patients who achieved CMR4 with imatinib therapy after diagnosed as new CP CML in Seoul St. Mary’s Hospital from 15Aug 2001 to 18 Dec 2009 were monitored by RQ-PCR assay at regular intervals to evaluate the molecular response. Patients’ median age was 43 (range 17 - 68) and median follow-up duration were 71 months (range 97 - 108.8 months) after commencing imatinib and 19,6 months (range 2,8 - 88,8 months) after achieving CMR4. Molecular response was expressed as a ratio of BCR-ABL to ABL% according to the international scale (IS), and CMR4, CMR4.5 and CMR5 are defined as 4-log, 4.5-log and 5-log reductions from the baseline, respectively. Results. We divided estimated level of CMR 0.0 - 5.0 in standard-ized baseline in our RQ-PCR assay, we could discriminate CMR4, CMR4.5 and CMR5, and different dynamics of molecular responses were demonstrated in each group as shown in Table 1.

Table 1. Molecular dynamics after achieving CMR4.0.

Among 58 patients with CMR4, 9 patients achieved CMR4.5, but not CMR5 (Group 1), 15 patients achieved CMR5, but not CMR4 (Group 2), and 31 patients obtained CMR5 (Group 3). All patients have maintained complete hematologic response (CHR), complete cytogenetic response (CCyR) and MMR without disease progression, and survival rate was 98.1% (52/53) with 1 patient died from complications after SCT. Although 7 patients showed fluctuating BCR-ABL levels under/across CMR4.0 levels, all of them maintained CMR4.5 without further progression to advanced disease. Conclusions. Although different molecular dynamics were observed after achieving CMR4, this study demonstrates that the achievement of CMR4 represents a stable level where the risk of progression including loss of MMR is extremely low. Analysis of molecular dynamics after achieving CMR4 examined here can be extended to expand our understanding of molecular profiles in CML patients through clinical application in a larger cohort with longer follow-up. With further follow-up, other possible significances of achieving CMR4 could be determined.

0153
DYNAMICS OF MOLECULAR RESPONSE TO STANDARD-DOSE IMATINIB IN NEW CP CHRONIC MYELOID LEUKEMIA PATIENTS AFTER ACHIEVING CMR4.0

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1The Catholic University of Korea, Seoul, South-Korea
2Seoul, South-Korea

Background. Imatinib has been the standard therapy in CML patients, and sustained undetectable BCR-ABL using real time quantitative polymerase chain reaction (RO-PCR) is often referred to as complete molecular response (CMR). With prolonged imatinib therapy, the number of patients with CMR are increasing, and an estimated 40% of chronic phase (CP) CML patients will achieve CMR after 5 years on imatinib therapy. However, the degree of CMR might be different in each laboratory depending on the sensitivity of RQ-PCR assays, and it is important to confirm what level of sensitivity is necessary to determine the stability of patients on therapy. Although the significance of achievement of major molecular response (MMR) has been extensively studies in the previous studies, according to our knowledge, significance of achievement of CMR has not been determined yet. Aims. In this study, we investigated the dynamics of molecular response to imatinib after achieving CMR4 in CP CML patients, and investigated their current clinical status to determine whether the achievement of CMR4 represents the level of stability in patients on imatinib therapy. Methods. Fifty three patients who achieved CMR4 with imatinib therapy after diagnosed as new CP CML in Seoul St. Mary’s Hospital from 15Aug 2001 to 18 Dec 2009 were monitored by RQ-PCR assay at regular intervals to evaluate the molecular response. Patients’ median age was 43 (range 17 - 68) and median follow-up duration were 71 months (range 97 - 108.8 months) after commencing imatinib and 19.6 months (range 2.8 - 88.8 months) after achieving CMR4. Molecular response was expressed as a ratio of BCR-ABL to ABL% according to the international scale (IS), and CMR4, CMR4.5 and CMR5 are defined as 4-log, 4.5-log and 5-log reductions from the baseline, respectively. Results. We divided estimated level of CMR 0.0 - 5.0 in standard-ized baseline in our RQ-PCR assay, we could discriminate CMR4, CMR4.5 and CMR5, and different dynamics of molecular responses were demonstrated in each group as shown in Table 1.

Table 1. Molecular dynamics after achieving CMR4.0.
acknowledgements and the European LeukemiaNet (ELN) based on previous studies.
Importance of MMR is obvious. However, results of studies regarding the
prognostic significance of complete molecular response (CMR) are
conflicting. Aims. To investigate the effect of CMR achieved by ima-
tinib mesylate (IM) on progression-free survival (PFS) in patients with
CML. Secondary aim of the study was to compare disease outcomes in
BCR-ABL positive and negative patients with MMR. Methods. Data from
106 CML patients receiving imatinib mesylate therapy were eval-
uated retrospectively. Of those, 35 patients were excluded because
MMR could not be achieved. Demographic and clinical data of the re-
mainin 71 patients are shown in Table 1. Remission definition and cy-
togenetic and molecular monitoring during the treatment was per-
formed in accordance with the criteria of the ELN. CMR was defined
as no detection of BCR-ABL by two consecutive polymerase chain re-
action analyses at least three months apart. Any treatment failure
was defined as progression. PFS was estimated as the time elapsed from
the beginning of the treatment until the progression or last contact. Overall
survival (OS) was calculated as the time between the beginning of the
treatment until death or last contact. Demographic data were com-
pared by descriptive statistics, χ², Student's t-test, and Mann Whitney
U tests. Survival analyses and curves were performed by log-rank test and
Kaplan-Meier method, respectively. Results. CMR was obtained in
59 out of 71 (83.1%) patients with MMR. Median PFS was not reached
in both BCR-ABL positive and negative groups. 3-year PFS was 74.1 %
and 89.8 % for BCR-ABL PCR positive and negative patients, respec-
tively (p = 0.3). Only one patient has died among 71 patients. 3-year OS
was 100% vs. 97.1% for BCR-ABL PCR negative and positive patients,
respectively (p = 0.7). There were no differences between groups re-
garding disease outcomes including treatment failure, disease transfor-
mation, and death (Table 2). Conclusions. Although this is a retrospec-
tive study including only a limited number of patients with CMR, our
results show that achieving CMR in patients with CML has no effect
on either PFS or disease outcomes. CMR should not be an essential goal
in the treatment of CML patients.

Table 1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>BCR-ABL PCR (-)</th>
<th>BCR-ABL PCR (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ± SEM) years</td>
<td>47±1.84</td>
<td>58±8.11</td>
</tr>
<tr>
<td>Female (%)</td>
<td>55 (33.3%)</td>
<td>31 (51.6%)</td>
</tr>
<tr>
<td>Sokal risk score (%)</td>
<td>17 (12.1%)</td>
<td>17 (27.0%)</td>
</tr>
<tr>
<td>Time since diagnosis (mo.)</td>
<td>14 (4-104)</td>
<td>19 (7-120)</td>
</tr>
<tr>
<td>Time to imatinib mesylate (%)</td>
<td>6 (2-35)</td>
<td>10 (4-120)</td>
</tr>
<tr>
<td>Time to first mmr (%)</td>
<td>18 (14-28)</td>
<td>29 (20-52)</td>
</tr>
<tr>
<td>BCR-ABL PCR (%)</td>
<td>10 (0-50)</td>
<td>15 (0-60)</td>
</tr>
<tr>
<td>Death (%)</td>
<td>5 (3.3%)</td>
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Table 2.

<table>
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<tr>
<td>Treatment failure (%)</td>
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LIVER IRON CONCENTRATION AND MORBIDITY IN PATIENTS WITH THALASSEMIA INTERMEDIA

K Musallam,1 J Wood,2 M Cappellini,1 I Motta,1 H Tamim,1 A Taher1
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2Children’s Hospital Los Angeles, Los Angeles, CA, United States of America
3University of Milan, Milan, Italy

Background. Patients with thalassemia intermedia (TI) can have significant iron overload, irrespective of transfusion status, secondary to increased intestinal iron absorption. Correlation between serum ferritin levels ≥1000 ng/ml and the rate of clinical complications in TI has been studied; however, data on the effect of elevated liver iron concentration (LIC) on morbidity (LIC) is lacking. Aims. To evaluate the association between LIC and several morbidities in patients with TI. Methods. A retrospective study of 168 TI patients treated at two centers in Beirut, Lebanon and Milan, Italy; for whom an LIC measurement was available. None of the patients were receiving iron chelation therapy. Data on demographics (age and gender), spleen size status, transfusion status, and presence of clinical complications were retrieved. Mean values of SF, fet, total hemoglobin (Hb), and total ferritin (Tf) levels, as well as platelet and nucleated red blood cell (NRBC) counts were retrieved. For LIC, direct determination of iron burden was performed using R2 MRI in Lebanon and T2* MRI in Italy using established methodology. Results. The mean age was 35.2 ± 12.6 years with 42.9% being males. A total of 121 (72%) patients were splenectomized and 44 (26.2%) were transfusion independent. The mean LIC was 8.4 ± 6.7 mg Fe/g dry weight (dw). On bivariate analysis, mean LIC values were significantly higher in patients with leg ulcers (P<0.05), thrombosis (P=0.01), pulmonary hypertension (P<0.01), abnormal liver function (P<0.01), hypothyroidism (P<0.05), osteoporosis (P<0.01), and hypogonadism (P<0.01) than those without. On multivariate logistic regression analysis, after adjusting for age, gender, spleen size status, transfusion status, and laboratory indices, a 1-mg Fe/g dw increase in LIC was independently associated with higher odds of thrombosis (AOR: 1.12, 95% CI: 1.05-1.20), pulmonary hypertension (AOR: 1.08, 95% CI: 1.02-1.14), hypothyroidism (AOR: 1.05, 95% CI: 1.01-1.11), osteoporosis (AOR: 1.08, 95% CI: 1.02-1.14), and hypogonadism (AOR: 1.10, 95% CI: 1.03-1.16). Summary/Conclusions. Elevated LIC is associated with vascular and endocrine complications in patients with TI.

EVALUATING PLATELET FUNCTION IN PATIENTS WITH THALASSEMIA USING THE PFA-100 SYSTEM; THE EFFECT OF CHELATION TREATMENT

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2Hemostasis Hemophilia Unit, Agia Sofia Children’s Hospital, Athens, Greece

Background. Patients with thalassemia are at increased risk for developing thrombotic and thrombocytopenic events. Prophylactic use of aspirin, though widely adopted in these patients, is mainly based on theoretical background. Furthermore, the effect of iron chelation therapy in platelet aggregation is not known. Aims. To evaluate the use of a screening method for platelet function in patients with thalassemia and to assess how the results of the method are affected by iron chelation therapy. Methods. Samples from the patients after informed consent was obtained, were evaluated using the PFA-100 platelet function analyzer (Dade Behring Inc. Deerfield, IL, USA). This instrument uses small membranes coated with either collagen and epinephrine (Col/Epi) or collagen and ADP (Col/ADP). Blood samples pass through the membranes at a high shear rate, simulating the in vivo hemodynamics in the small capillaries. The PFA test is considered a rapid, accurate screening test to measure both platelet adhesion and aggregation (primary hemostasis). Sixteen patients (10 females, mean age: 33 ± 6.4 years) followed in our unit with either thalassemia major (12 patients) or thalassemia intermedia (4 patients) were included in the study. Patients were on chelation treatment with either deferoxamine (DFO) (2 patients), deferiprone (5 patients) or combination therapy of DFO and deferiprone (5 patients). Fourteen patients underwent a 2nd evaluation, 2-4 weeks after starting or combination therapy of DFO and deferiprone. Results. Mean PFA-100 levels at the first evaluation were 114.9 ± 15.5 sec, which were within the reference range. Values were not significantly different according to gender or type of thalassemia. After initiation of therapy with deferasirox there was a significant increase in the PFA-100 levels from a mean value of 113 ±12.9sec to 132.6 ± 23.1 (P<0.05). In 6 of these patients the Col/Epi closure time was longer than the reference value with deferiprone, showing also prolongation of the Col/ADP closure time, indicating a more profound effect on platelet function. The prolongation of Col/Epi closure time parallels the changes in renal function, a well described phenomenon observed during treatment with deferasirox (P<0.05). Conclusions. A significant change was noted in the results of the PFA-100 evaluation after initiation of treatment with deferasirox. A possible mechanism for this phenomenon may be the inhibition of the heme-containing enzyme cyclooxygenase of the platelets, which produces thromboxane A2 from arachidonic acid, a potent regulator of platelets aggregation. This observation may have significant clinical applications for in patients receiving deferasirox. Toxicity from the chelator may be prevented by avoiding other drugs which have similar effect to platelets aggregation. Moreover, the need for concomitant antithrombotic prophylaxis with aspirin needs to be reconsidered. Larger long-term studies are required to validate these observations.
A Meloni,1 V Postiano,1 D De Marchi,1 P Kelberg,2 G Palazzi,3 L Pitrolo,3 G Giannotti,4 A Quota,5 S Macchi,5 M Smacchia,6 M Salvatori,6 M Lombardi,1 A Pepe1

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0160 ONSET OF CARDIAC IRON LOADING IN A LARGE AND HOMOGENOUS COHORT OF THALASSEMA MAJOR PEDIATRIC PATIENTS

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4 Clin. di Emato-Oncologia Pediatrica, Universita/Azienda Ospedaliera, Padova, Italy

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Background. Heart failure remains the main cause of mortality in thalassemia major (TM). Magnetic Resonance Imaging (MRI) by the T2* approach is the unique non invasive technique for highly reproducible quantifications of myocardial iron burden and is the gold standard for quantifying biventricular function parameters. It is important to determine the appropriate age to start MRI screening, because its high cost and no large availability. Few data are available in the literature. Aims. The aim of this study was to address this issue in a cohort of paediatric patients selected from a large TM population homogenous for geographic origin and treatment. Methods. We studied retrospectively 72 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network with an age < 18 years (47 males, 42.7-17.9 years old, mean age 13.0±3.7 years). Myocardial iron overload (MIO) was measured by T2* multislice multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. Results. The global heart T2* value was 29±11 ms. No MIO was detected in the 33% of the patients; 44% of the patients showed a heterogeneous MIO with a global T2* value > 20 ms; 10% showed a heterogeneous MIO with a T2* global value < 20 ms and 15% had a homogeneous MIO. No significant correlation was found between global heart T2* value and age, OR for a global heart T2* < 20 ms was 1.13 (P = 0.18) for boys and 1.2 (P = 0.05) for girls. The mean age when the T2* value > 20 ms was 12.3±3.3 years. Conclusion. The MRI screening for both iron overload and function assessment can be started for TM patients at the age of 12 years. At this age not sedation is generally needed. If the availability of cardiac MRI is low, the serum ferritin levels can be used as a discriminating factor.
fit had a slope of 1.917±0.061 and an intercept of -9.145±13.083 Hz. The R-squared value for the fit was 0.975. Figure 1B shows the global heart T2* values at 3T plotted against the corresponding values at 1.5T for the patients. The line of best fit for the patients had a slope of 1.961±0.053 and an intercept of -17.570±3.160 Hz. The R-squared value for the fit was 0.984. Specific conversion formulas for liver and global heart T2* values at 3T vs T2* at 1.5T are indicated in Figure 1C. Conclusion. For both liver and heart, the T2* values at 3T were about twice that at 1.5T with an intercept depending on the non-iron component of the tissue. A conversion formula between T2* values at 3T and 1.5T was introduced and it may be used in the clinical arena by the centers that use 3T scanners.

Figure 1.

**0162**

DIFFERENT PATTERNS OF MYOCARDIAL IRON OVERLOAD BY MULTISLICE T2* CARDIOVASCULAR MR AS MARKERS OF RISK FOR CARDIAC DYSFUNCTION IN THALASSEMIA MAJOR

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Introduction. The multislice multiecho T2* Cardiovascular Magnetic Resonance (CMR) technique allows to detect different patterns of myocardial iron overload (MIO). Aims. The aim of this study was to verify the role of biventricular dysfunction related to different patterns of MIO in a large cohort of thalassemia major (TM) patients. Methods. 1135 TM patients (538 M, 30±19 years) enrolled in the Myocardial Iron Overload in Thalassemia (MIOT) network underwent CMR. For the assessment of MIO, three short-axis views of the left ventricle (LV) were acquired and the myocardium was segmented into 16-segments standardized LV model. The T2* value on each segment was calculated as well as the global T2* value. Biventricular functional parameters were quantitatively evaluated by cine images. Results. Four groups of patients were identified: homogeneous MIO (all segments with T2*<20 ms) (N=175), heterogeneous MIO (some segments with T2*≥20 ms and other segments with T2*<20 ms) and global heart T2*<20 ms (N=160), heterogeneous MIO and global heart T2*≥20 ms (N=33) and no MIO (all segments with T2*>20 ms) (N=465). The LV ejection fraction (EF) was significant different among the groups (P<0.0001) (Figure 1, top). Odds Ratio for LV dysfunction (LV EF<57%) was 4.8 (1.7-7.3 OR 95%CI; P=0.001) for patients with homogeneous MIO and global heart T2*<20 ms and other segments with T2*<20 ms and global heart T2*≥20 ms (N=160), heterogeneous MIO and global heart T2*≥20 ms (N=33) and no MIO (all segments with T2*>20 ms) (N=465). The LV ejection fraction (EF) was significant different among the groups (P<0.0001) (Figure 1, top). Odds Ratio for LV dysfunction (LV EF<57%) was 2.1 (1.4-3.2 OR 95%CI; P=0.001) for patients with homogeneous MIO vs patients with no MIO. Right ventricular (RV) EF was significant different among the groups (P<0.0001) (Figure 1, bottom). Odds Ratio for RV dysfunction (RV EF<55%) was 4.1 (1.4-11.2 OR 95%CI; P=0.001) for patients with homogeneous MIO vs patients with no MIO. Conclusion. Biventricular dysfunction is correlated with MIO distribution decreasing from the patients with homogeneous MIO to the patients with no MIO. Homogeneous MIO and heterogeneous MIO with a global heart T2*<20 preddicts a significantly higher risk to develop cardiac dysfunction suggesting an intensive chelation therapy in this group of patients.

Figure 1.

**0163**

THYROID DYSFUNCTION IN PEDIATRIC EGYPTIAN THALASSEMICS: WHAT ABOUT IODINE DEFICIENCY?

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Introduction. Primary hypothyroidism is one of the most frequent endocrinological complications observed in patients suffering from thalassemia (10-50%). Long standing anemia, hypoxia and hemosiderosis were proposed as a cause. Iodine deficiency is an important contributing factor for hypothyroidism. Iodine deficiency is not uncommon among Egyptian children, its contribution to hypothyroidism in thalassemia major patients was not studied. The urinary iodine concentration is the most useful test for assessing iodine nutrition in populations. The aim of the present study was to assess the thyroid hormones and the urinary iodine level in Egyptian thalassemic pediatric patients. Study design. Sixty thalassemia patients (31 males, 29 females), mean age 14.4±3.3 years) followed up at the hematology clinic were randomly selected to participate in this study together with thirty six age and sex matched controls after the parents gave their consent. All patients were subjected to thorough clinical examination. Serum evaluation of T3, T4, TSH and urinary iodine. Results. A highly significant difference was observed in urinary iodine, T3, T4 and TSH between the thalassemic and the control groups (P<0.001). T3 was low in 51.7% (n=49), T4 in 68.3% (n=41) while TSH was elevated in 81.7% (n=49) of the patients. Overt hypothyroidism (low T4 and elevated TSH >10 ulU/ml) was present in 45% (n=27) of the patients, while all controls were euthyroid with normal urinary iodine. Severe iodine deficiency was present in 15% (n=9), moderate deficiency in 45% (n=27) and mild deficiency in 40% (n=24) of the patients. Thyroid function tests results varied among these groups of iodine deficiency. A negative correlation was found between urinary iodine and both serum ferritin and TSH (r=-0.413, P<0.001, and r=0.881, P< 0.001 respectively) and both serum ferritin and age (r=0.385, P<0.001, r=0.491, P<0.001). Conclusions. Correction or supplementation of iodine to thalassemia patients may have a role in prevention or correction of hypothyroidism in thalassemia patients especially in areas where iodine deficiency is common like Egypt.
A MRI PROSPECTIVE SURVEY ON HEART AND LIVER IRON AND CARDIAC FUNCTION IN THALASSEMAIA MAJOR PATIENTS TREATED WITH DEFERASIROX VERSUS DEFERIPRONE AND DESFERRIOXAMINE IN MONOTHERAPY

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Background. No prospective data are available about the efficacy of deferasirox versus deferiprone and desferrioxamine in monotherapy. Aims. Our study aimed to prospectively assess the efficacy of deferasirox versus deferiprone and desferrioxamine in monotherapy in a large cohort of thalassaemia major (TM) patients by quantitative Magnetic Resonance (MR). Methods. Among the 1135 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network we selected those with an MR follow up study at 18±3 months who had been received one chelator alone between the 2 MR scans, We identified three groups of patients: 80 treated with DFX, 59 with DPF and 74 with DFO. Iron overload was measured by T2* multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. Results. Excellent/good levels of compliance were similar in the DFX (98.8%) vs DFP (94.9%) and DFO (95.9%) groups. The percentage of patients that maintained a normal left ventricular ejection fraction (LVEF≥57%) was comparable for DFX (98.1%) vs DFP (100%) and DFO (98.1%) groups. Among the patients with myocardial iron overload at baseline, in all three groups there was a significant improvement in the global heart T2* value (DFX: +3.5±4.7 ms P=0.001, DFP: +8.3±8.6 ms P=0.015 and DFO: 3.7±5.5 ms P=0.001) and a reduction in the number of pathological segments (DFX: -2.4±3.8 P=0.003, DFP: -6.0±5.6 ms P=0.012 and DFO: -2.9±3.7 ms P=0.001).

*P<0.05, **P<0.01, ***P<0.001

Table 1.

<table>
<thead>
<tr>
<th>Category</th>
<th>Age 1-2</th>
<th>Age 12-19</th>
<th>Age 20 &gt;</th>
<th>Serum ferritin (µg/L) ≥ 200</th>
<th>Measured age ≤ 1</th>
<th>Other age</th>
<th>Transfusion age ≥ 2</th>
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<td>6.8</td>
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<td>2.8</td>
<td>4.0</td>
<td>4.3</td>
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<tr>
<td>Category 3</td>
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<td>8.4</td>
<td>3.0</td>
<td>4.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Only in the DFP group there was a significant improvement in left and right ventricular ejection functions (≤5-6.4% P=0.045 and +6.8±3.7% P=0.001, respectively). The improvement in the global heart T2* was significantly lower in the DFX versus the DFP group (P=0.026, see figure), but it was not significantly different in the DFX versus the DFO group. The improvement in the LVEF and in the RVEF was higher in the DFP group than in the DFX group at the limit of the significance (P=0.066 and P=0.062, respectively), it was comparable between DFX and DFO groups. Among the patients with hepatic iron at baseline (T2*<9.2 ms) the changes were not significantly different in DFX versus the other groups. Conclusions. prospectively in a large clinical setting of TM patients, DFX monotherapy was significantly less effective than DFP in improving myocardial siderosis and in maintaining a normal left ventricular ejection fraction.

THE PALATABILITY AND TOLERABILITY OF DEFERASIROX TAKEN WITH DIFFERENT LIQUIDS OR FOOD

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Background. Deferasirox is an oral iron chelator indicated for the treatment of transfusional hemosiderosis in patients aged ≥2 years. The recommended mode of administration is to be taken on an empty stomach in water, apple juice or orange juice ≥30 minutes before food. Post-marketing reports have noted discontinuation or reduced compliance of deferasirox secondary to palatability and gastrointestinal adverse events. Registration trials with deferasirox did not evaluate food combinations to maintain predictable plasma levels since single dose pharmacokinetic studies suggested that bioavailability was increased by food. Aims. Assess palatability and tolerability of deferasirox when taken with meals, various liquids, and crushed and added to food. Methods. A single-arm, open-label, multi-center study (NCT00845871) enrolling patients with transfusional hemosiderosis (minimum entry serum ferritin ≥200 µg/L) aged ≥2 years with thalassemia major, sickle cell disease, low or intermediate-1 risk MDS or other anemias, who were on, starting, or resuming treatment with deferasirox. The study began with a 1-month run-in phase with deferasirox dosed according to prescribing information. Subjects then had the option of taking deferasirox with/without meals, morning/evening, or intermediate-1 risk MDS or other anemias, who were on, starting, or resuming treatment with deferasirox. The study began with a 1-month run-in phase where subjects chose their recommended mode of administration. There were 5 options: with/without meals; morning/evening; intermediate-1 risk MDS or other anemias, who were on, starting, or resuming treatment with deferasirox. The study began with a 1-month run-in phase where subjects chose their palatability and tolerability of deferasirox when taken with meals, various liquids, and crushed and added to food. Methods. A single-arm, open-label, multi-center study (NCT00845871) enrolling patients with transfusional hemosiderosis (minimum entry serum ferritin ≥200 µg/L) aged ≥2 years with thalassemia major, sickle cell disease, low or intermediate-1 risk MDS or other anemias, who were on, starting, or resuming treatment with deferasirox. The study began with a 1-month run-in phase with deferasirox dosed according to prescribing information. Subjects then had the option of taking deferasirox with/without meals, morning/evening; crushed and added to soft food/liquid of choice. Palatability was assessed with a Five-Point Facial Hedonic Scale with additional questions capturing GI effects. Results. Sixty-five patients were enrolled (Table 1).
Palatability ratings improved with the introduction of food. The majority of patients (84%) rated a mode as “dislike extremely” or “somewhat dislike” was lower during the assessment phase as compared to the run-in phase. No particular mode was clearly more palatable than others. The most popular soft foods chosen were apple sauce and yogurt; the most popular liquids chosen were orange and apple juice. An age effect was observed for GI disturbances during the (fasting) run-in phase with older subjects (≥60 years) reporting higher rates of nausea (40% versus 9.6%, P=0.04) and diarrhea (50% versus 19%, P=0.09) compared to younger subjects. Gastrointestinal adverse events decreased with the addition of food. There were 46 occurrences of diarrhea in 15 patients (n=62) during run-in versus 29 occurrences in 7 patients during assessment (P=0.08 by patients); there were 2 patients with diarrhea in both the run-in and assessment phases. Most diarrheal events occurred ≤2 hours after dose. The rates of other GI events in the run-in versus assessment phases included: 54 versus 47 abdominal pain events; 31 versus 23 nausea events; and 10 versus 15 vomiting events. There were no marked differences in renal or liver function tests between phases. Conclusions. Administration of deferasirox with food was rated by patients as more palatable and appeared to lessen GI adverse events. This study suggests that the addition of food may improve the palatability and tolerability of deferasirox among those experiencing difficulties with the approved dosing schedule. Further evaluations of safety and the effect of food on pharmacokinetics at steady state are required.

0166
INCIDENCE OF ARTERIAL AND VENOUS THROMBOEMBOLIC EVENTS AMONG PATIENTS WITH THALASSEMIA. ANALYSIS OF PREDISPOSING FACTORS
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Background/Aims. Homozygous beta-thalassemia has been considered a hypercoagulable state, particularly following splenectomy. However, few large series of patients have been reported, describing the frequency of thrombotic complications in this group of diseases. We herein present our experience on venous and arterial thrombotic events, encountered among a large group of 585 thalassemic patients and investigate the relevant predisposing factors. Patients and Methods. All 585 thalassemic patients of three Thalasemia Units were studied, and all venous and arterial thromboembolic events were collected and analyzed. Three hundred and sixty-one patients (67.1%) had thalassemia major (TM), 168 (31.2%) had thalassemia intermedia (TI), and 9 had Hemoglobin (Hb) H disease (1.7%). In this population the prevalence of thromboembolic events was analyzed, in relation to disease genotype, patient’s age, frequency of transfusions, serum ferritin levels, type of iron chelation, the presence of HCV infection, adequacy of hepatic function, history of previous splenectomy, Hb levels, MCV, platelet count, mean platelet volume (MPV), presence of nucleated red blood cells (RBC), total serum globulin levels, and the presence of FV Leiden, FII 20210A, beta-FLAG-nogen-455C>G, A2α, FVII-, MTHFR C677T and MTHFR F1298C mutations, which have been associated with a hereditary thrombophilic state. Results. Totally, 17 (3.2%) venous and 7 (1.3%) arterial thromboembolic events were recorded. In particular, there were 7 episodes of portal vein thrombosis, 4 of pulmonary embolism, 4 of pulmonary hypertension, 3 of central venous catheter thrombosis, extended to left atrium, 3 of deep vein thrombosis (DVT) and 3 ischemic cerebral attacks. The frequencies of thrombophilic mutations in the studied population were: 2.5% for FV Leiden, 1.7% for FII 20210A and 7.1% for MTHFR C677T polymorphism homozygotic. These mutations were not related to any increase in the frequency of thromboembolic events in TM and TI. Statistical analysis of the remaining parameters studied, revealed that genotype IVS-6, and previous splenectomy were independent risk factors for the appearance of both, venous and arterial thrombosis. The prevalence of venous thrombosis in TM and TI were 2.49% and 7.7% respectively, whereas the prevalence of arterial thrombosis was estimated at 0% for TM and 4.2% for TI. By removing TI and arterial thromboembolic events in TM and TI, the prevalence of venous thrombosis in TM was calculated 1.66%. Fourteen of the 17 venous thrombotic episodes and all arterial thrombotic events occurred in splenectomized patients. These events occurred 8 days to 45 years post-splenectomy. Platelet count at the onset of thrombotic event ranged between 300,000/μl and 1,400,000/μl, but was not a significant factor for the event. TI patients with a thrombotic episode were sporadically transfused, while they were splenectomised due to severe hypersplenism. Conclusions. TI patients exhibit substantially higher risk for both, venous and arterial thrombosis as compared to TM patients. Splenectomy and disease genotype, associated with an intermediate phenotype were shown to be the major determinants for thrombosis. For TI patients, not regularly transfused, the presence of abnormal and of nucleated RBCs in circulation and endothelial damage are considered major factors contributing to thrombosis.

0167
NEW INSIGHTS ON ß-THALASSEMIA IN GAZA: HIGH FREQUENCY AND Milder PHENOTYPE AMONG IVS1-1 HOMOZYGOUS PATIENTS WITH HIGH LEVELS OF FETAL HEMOGLOBIN
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Background. Beta thalassemia is a very common disease in the Gaza strip. The rate of carriage is estimated to be 4.5% and there are about 500 homozygous patients treated by transfusions. Premarital screening is being implemented since 2000 and this is the only mean of prevention as prenatal diagnosis is not performed routinely. The aim of the present study is to understand better the correlation between genotype and phenotype in the thalassemic population of Gaza. Patients and Methods. DNA was sampled from 68 patients with TM and 48 patients with TI, while 49 healthy controls were also sampled. Genotyping was performed using PCR followed by either Allele Specific Oligonucleotide hybridization, sequencing and endonuclease restriction. Results. Thirteen different mutations have been identified, which have been previously described in Mediterranean populations. However, the distribution of the mutated alleles among the patients in Gaza is quite unique. The most common mutation was IVS-1-1G-A, which was prevalent in 31.5% of the thalassemic alleles. The IVS-1-110 mutation, which is the most frequent mutation in the Eastern Mediterranean as well as in other Arab countries, was found in 25% of alleles. In addition, the mutations of IVS1-6, Frameshift 5, Nonsense 39, IVS1-110 were found among 9%, 8%, 7% and 6% of the chromosomes respectively. Two rare mutations were also found. The novel Poly A mutation AATAAA-AAAA, which was reported previously only in Gaza, was found in an additional 4 patients. Additionally, the 28 bp deletion which is prevalent in the Arab Gulf countries, mostly in Bahrain, was first diagnosed in a Palestinian family. Fifteen patients were homozygotes for IVS1-1 and 20 were homozygotes for IVS1-110 mutations. The homozygotes for IVS1-11 mutation required less blood, since they consumed only 13 packed red cell units a year while those with IVS1-110 consumed 24 blood units a year (P=0.001). Their mean hemoglobin level was 8.3gr/dl and 7.68gr/dl (Figure A,B) respectively, and there was no significant difference in age or gender distribution between these two groups. The reason for the difference in blood requirements between the 2 groups are over expression of fetal hemoglobin (HbF). The mean HbF level was 46% in the homozygotes for IVS 1-1 and 6% for those with IVS 1-110 (p<0.0001) (Figure C). The XmnI polymorphism at the gamma globin gene, which is known to be associated with elevated HbF levels, was found in 7 out of 15 IVS1-1 patients. The XmnI positive patients had HbF level of 76.86% compared to 18.76% (p<0.0001). Conclusion. The distribution of ß-thalassemia among patients in Gaza is unique with a highest frequency of IVS-1-11 mutation which is a β0 mutation resulting in severe β-thalassemia major. Counter to expectation, these patients required less blood transfusions, due to persistent production of HbF. The association between IVS 1-1 mutation with high expression of HbF has not been previously described.
0168
MATERIAL AND FETAL COMPLICATIONS IN THALASSEMAIA MAJOR: OUTCOMES FROM A 10 YEAR STUDY AT MOUNT SINAI HOSPITAL IN TORONTO, CANADA
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Background. Thalassemia major is an inherited disorder of ineffective erythropoiesis, resulting from severely defective or absent production of beta globin chains. Lifelong chronic transfusion support leads to iron overload, affecting the pituitary-gonadal axis as well as cardiac and hepatic disease. While advances in the detection of iron overload and improved chelation therapy, it has been increasingly possible for women to successfully undergo pregnancy. This is the first centre in North America to date that has studied pregnancy outcomes in this population. Aims: To identify maternal and fetal complications in pregnant women with Beta Thalassemia Major, with reference to the Ontario population. Methods. A retrospective study of consecutive deliveries of thalassemia major patients at Mount Sinai Hospital Special Pregnancy Program, from September 2000 through December 2010 was conducted. Maternal demographics, pregnancy complications including transfusions, mode of delivery, apgar scores and fetal complications were recorded. Patients were identified from the Special Pregnancy Database and the Delivery Databases of Mt. Sinai Hospital. Electronic patient records from the Toronto General Hospital and Mt.Sinai Hospital paper charts were reviewed. Results. A total of 44 women were identified. There were 41 singleton and 2 sets of twin live birth deliveries and 1 neonatal death. Median maternal age was 32(range 21 to 41) years with a median gestational age of 38.5 (range 27 to 41) weeks. Only 15% of women delivered before 37 weeks. For 56% of women it was their first live birth. 39% of deliveries were by C-section. One mother developed mirtal regurgitation during the pregnancy. Gestational diabetes developed in 9% and pregnancy induced hypertension (PIH) in 24/44 (4.5%) There were no cases of intra-uterine growth retardation. Median birth weight was 3158 (980-4860). 13% of neonates were born less than 2.5 kg. Mean apgar scores at 1 and 5 minutes were 8.5 ± 1.5 and 9.0 ± 0.2 respectively. There were no fetal anomalies. Summary/Conclusions. Although the C-section rate was higher than the 2005/2006 Ontario rate of 28% it was at the lower end of the reported range in Thalassemia, of 26-93%. This relatively low C section rate probably reflects optimal transfusion support and suppression of marrow expansion, preserving normal pelvic anatomy. Preterm labour and neonates with a low birth weight were higher than the 2005/2006 Ontario rates of 5.6% and 7% respectively. Gestational diabetes was higher than the 4% found in the Ontario data, indicating pituitary and pancreatic iron deposition. Other maternal and fetal outcomes in our study did not differ from averages for healthy women. Pregnancy outcomes in women with thalassemia major are excellent if care is provided by an experienced multidisciplinary team including hematologists and high risk pregnancy obstetricians.

0169
RELATIONSHIP BETWEEN SERUM FERRITIN AND LIVER IRON CONCENTRATION IN HEMOGLOBIN E THALASSEMIA
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Background. Hemoglobin E thalassemia, the most common form of severe thalassemia worldwide, is associated with body iron loading even in the absence of frequent transfusions. Aims. The aim of the study was to determine the degree of relationship between serum ferritin and liver iron concentration in hemoglobin E thalassemia patients. Methods. Approvals were obtained from the human research ethics committees at The University of Kelaniya, The University of Western Australia, and the University Health Network, Toronto. Informed consent was obtained from all subjects. Hemoglobin E thalassemia subjects were recruited from the National Thalassaemia Centre, Kurunegala, Sri Lanka. Patients were then categorized into to two groups (1) those who had received less than or equal to 20 transfusions lifetime (N=25) and (2) those who had received more than 20 transfusions lifetime (N=61), LIC was measured for all subjects using spin density projection assisted R2-MRI (FerriScan®). Results. The mean age of subjects was 27.3 ± 16.1 [range 7.8 to 57.4] years for group 1 and 23.5 ± 10.1 [range 8.5 to 60.2] years for group 2. The figure shows the relationships between serum ferritin and LIC for groups 1 and 2. Solid lines are linear regression fits to the data; gradient 29.9 (± standard error 10.1) ng ferritin/L per mg Fe/g dw for group 1 and gradient 71.0 (± standard error 15.1) ng ferritin/L per mg Fe/g dw for group 2. Dashed lines represent the 95% confidence bands of the line of best fit. For group 1, there was a weak (r=0.28) but significant (p=0.007) correlation between serum ferritin and LIC. For group 2, there was also a weak (r=-0.27) but significant (p< 0.001) correlation with LIC. However, for both groups the 95% prediction intervals for LIC for a given serum ferritin were so broad as to make serum ferritin of little clinical value in determining liver iron loading. For example, a serum ferritin of 1500 ng/L was associated with 95% prediction intervals of LIC of 21.2 to 83.1 mg Fe/g dw (group 1) and 0.5 to 15.2 mg Fe/g dw (group 2). Conclusions. Serum ferritin measurements have limited clinical value for assessing the degree of iron loading of patients with hemoglobin E thalassemia.

0170
PUBERTAL EVALUATION OF FEMALES AND MALES WITH B-THALASSEMIC MAJOR IN RELATION TO CHELATION REGIMEN
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While transfusion and iron chelation therapy have increased longevity of patients with beta-thalassemia major (BTM), yet puberty disorders and infertility have become more apparent. Aim of the study. To evaluate the prevalence of delayed puberty and the pituitary gonadal axis in transfusion-dependent BTM patients in relation to chelation type and degree of iron overload. Also, to assess the spermatogenetic function in pubertal males with BTM males. Patients and Methods. A two year prospective study of 95 regularly transfused thalassemics; 42 males and 51 females; 14 years and above. Sixty-Two (66.7%) were on deferiprone (DFP), 21 (24%) on desferrioxamine (DFO), and 10(9.3%) on combination DFO & DFP. At study entry, after baseline compliance analysis, endocrinal evaluation were performed. Those with delayed and arrested puberty were tested for pituitary-testicular axis and pituitary-ovarian function by gonadotrophin-releasing hormone (GnRH) and human chorionic gonadotropin (HCG) tests. Spermiograms were done for pubertal males. Patients with delayed puberty with good pituitary and gonadal responses were shifted to combined chelation therapy, and yearly pubertal assessment and spermiogram for pubertal males. Results. Median age was 20 years. Thirteen male patients (30.9%) had normal puberty response; they were on either DFP (75.8%), DFO (21.5%) or combination 4(30.8%), while twenty- nine (69%) had puberty disorder (21 on DFP, 8 on DFO); Out of non pubertal males; 19(44.3%) had good pituitary and testicular response to LHRR stimulation. Forty seven % of studied females had pubertal disorders; delayed puberty (17.6%), primary amenorrhea (17.6%) or secondary amenorrhea (15.7%) and 75% of the delayed puberty were on DFO alone. During two years follow-up using intensive combination chelation therapy(DFP + DFO), 7 of good pituitary responders males progressed to puberty and six females had reversal of their hypogonadism (24.9%). Total sperm count and sperm motility were decreased in 4 (25%) and 6 (35.3%) patients respectively. In conclusion, The risk of hypogonadism in B-TM patients remained high. Intensive combination chelation for 2 years lead to better sexual development in both sexes.

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HEART T2* FOR PREDICTION OF CARDIAC COMPLICATIONS IN WELL-TREATED TM PATIENTS

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Aims. The aim of this study was to establish prospectively the risk of cardiac complications in a large cohort of well-treated TM patients. Methods. We considered 527 TM patients (252 males, mean age 30±9 years) for who clinical data relative to a period of 5 years after the first MRI were collected in a central data base. At time of the first scan mean ferritin levels where 1653±1559 ng/l, global heart T2* was 22±13 ms, and excellent/good level of compliance were present in the 96% of the study population. Results. At 5 years of follow-up, we recorded 24 cardiac events: 4 episodes of cardiac failure, 15 of arrhythmia, 1 of pulmonary hypertensión and 4 of other cardiac complications. The majority of these events (21/24) happened within the first 24 months subsequent to the MRI, so we considered this follow-up period. At the first MRI scan, in patients with cardiac complications the global heart T2* was 22.5±12.4 ms. In comparison with global heart T2* values ≥20 ms, there was not a significantly increased risk of cardiac complications associated with global heart T2* values <20 ms (HR=2.085 P=0.09). In the heart failure patients the global heart T2* was 19±12 ms. In comparison with global heart T2* values ≥20 ms, there was not a significantly increased risk of heart failure associated with global heart T2* values <20 ms (HR=1.9 P=0.524) or <10 ms (HR=2.6 P=0.485). In the arrhythmia patients the global heart T2* was 25±13 ms. In comparison with global heart T2* values ≥20 ms, there was not a significantly increased risk of arrhythmia associated with global heart T2* values <20 ms (HR=2.1 P=0.179) or <10 ms (HR=0.8 P=0.824). During the follow up changes in the chelation therapy (type and/or dose-frequencies) were found in >25% of the study population. Conclusions. We detected very few cardiac events, almost all concentrated in the first 24 months. In a large cohort of well-treated TM patients heart T2* lost its power in predicting cardiac events probably due to a patient-specific adjustment of the chelation therapy MRI-guided.

Figure 1.
**Clinical thrombosis**

**0173**

**SELECTION CRITERIA OF PATIENTS WITH VENOUS THROMBOEMBOLISM FOR LABORATORY INVESTIGATION OF INHERITED THROMBOPHILIA**

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**Background.** Laboratory investigation for inherited thrombophilia is warranted in young patients, especially those with severe venous thromboembolism (VTE) occurred spontaneously or recurrently. Investigation of older patients is discouraged, especially when events are mild or provoked. Such policy could miss a number of carriers, leaving undiagnosed their kindreds. **Aims.** To investigate whether clinical parameters are predictive of the presence of inherited thrombophilia in VTE patients. **Patients and Methods.** We analyzed the files of 1,835 patients referred to our Thrombosis Center between 1996 and 2009. The median age at the first VTE was 37 years (range 0-89); 736 were males (40.1%). Patients were stratified according to family history of VTE, age of first VTE (<45 years), type of first VTE (defined severe for proximal DVT and/or pulmonary embolism and mild for distal DVT or superficial vein thrombosis), circumstances of first VTE (unprovoked or provoked), history of recurrent VTE. Multiple regression was carried out labelling as dependent variable diagnosis of overall thrombophilia or severe thrombophilia (antithrombin or protein C or protein S deficiency, homozygous or multiple defects, n=211) or mild thrombophilia (heterozygous factor V Leiden or prothrombin G20210A, n=415). **Results.** Diagnosis of overall thrombophilia was associated with family history (p=0.005), severity of VTE (p=0.008) and recurrent events (p=0.0001), the aforementioned criteria were all absent only in 8% of patients with thrombophilia. **Conclusions.** Family history, clinical severity and recurrence of VTE are strong predictors of inherited thrombophilia, and at least one of these parameters is present in more than 90% of cases. Selection of the patients to be investigated according only to age and/or circumstances of the first VTE could miss diagnosis of thrombophilia in a relevant number of cases.

**0174**

**THE IMPACT OF VENOUS THROMBOEMBOLISM IN CRITICALLY ILL PATIENTS: A META-ANALYSIS OF MAJOR CLINICAL OUTCOMES**

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**Purpose.** To systematically review whether a diagnosis of DVT in critically ill patients affects clinically important outcomes including length of stay, duration of mechanical ventilation and mortality. **Methods.** We carried out a comprehensive literature search of the English language literature. The following databases were searched: MEDLINE and EMBASE, and their subsets. Relevant references were identified by examining the reference lists of retrieved reports. **Results.** Nineteen prospective studies were included in the systematic review. Patients diagnosed with DVT compared to those without DVT had increased ICU and hospital stay (7.3 days (95% CI 1.4 to 13.2; F= 0.02) and 16.5 days (95% CI 1.51 to 50.59; P= 0.03), respectively. Duration of mechanical ventilation appeared to be increased in patients with DVT although this difference was not statistically significant (weighted mean difference: 3.41 days (95% CI 0.74 to 5.19; P = 0.17). **Conclusions.** A diagnosis of DVT upon ICU admission appears to affect clinically important outcomes including length of ICU and hospital stay and hospital mortality. Further research involving larger prospective study designs are warranted.

**Table 1.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value (95% CI)</th>
<th>p-value</th>
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<td>&lt;0.0001</td>
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<tr>
<td>Hospital stay</td>
<td>7.3 (1.4 to 13.2)</td>
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<tr>
<td>Mechanical ventilation</td>
<td>3.41 (0.74 to 5.19)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

**0175**

**VENOUS THROMBOEMBOLISM IN ADOLESCENTS WITH CANCER - A SINGLE CENTRE EXPERIENCE**

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**Background.** The association between cancer and venous thromboembolism (VTE) is well documented with a reported incidence of 4.1% from a large adult series. Risk factors for thrombosis include the underlying neoplasm and its therapy, patient age, other predisposing medical conditions and immobilization. Younger age is considered protective. The presence of central venous catheters (CVC) is perhaps the single most important predisposing factor in the development of VTE in children. The British Committee for Standards in Haematology (BCSH) has recently released a guideline on the prevention, investigation and management of VTE in children below 16 years of age. In March 2010 the NICE VTE thromboprophylaxis guidelines for adults aged 18 and above were introduced. Our adolescent unit treats approximately 72 teenagers (aged 13-19) with cancer each year, most of whom have CVCs inserted to facilitate treatment. There is a paucity of evidence for the risk of developing a VTE across this specific age range and lack of clarity in recommendations for patients between 16-18 years of age. This has prompted an evaluation of the VTE episodes in our unit and to audit our procedure against both current national guidelines. **Aims.** 1) To identify incidence of VTE in an adolescent cancer population and any specific patient characteristics and risk factors. 2) Audit our population and current practice...
against both sets of guidance. 3) Confirm our diagnostic methods and treatment follow national guidelines. Methods. Retrospective case note review of all adolescents with cancer diagnosed between 2005 and 2010 who developed a clinically significant VTE during treatment. Patient records were cross referenced with pharmacy records of patients treated with dalteparin (treatment of choice in our unit). Data were collated on a proforma and entered onto an electronic database. Results. 483 patients were treated in our unit between 2005 and 2010. 50/483 developed VTE during the course of their treatment (11.6%). 29/50 episodes were in girls, and 21/50 in boys. At least 21 VTE were line-associated. 7 patients had sagittal sinus thromboses of which 5 were contemporaneous to L-asparaginase. L-asparaginase administration was also associated with 11 other VTE (line-related, lower limb DVT and PE). Other notable risk factors include immobility due to surgery in 4 patients. Of note, one patient who was appropriately risk assessed pre-surgery (managed with graduated compression stockings and dalteparin) subsequently developed a line-associated VTE. There was a high degree of compliance with diagnostic techniques and therapy as recommended by both the BSH and NICE guidelines. Summary/Conclusions. Adolescents with cancer are at significant risk of developing VTE; in particular female patients with in-dwelling CVGs. Our incidence of VTE is higher than reported in adult patients with a cancer diagnosis. At the very least, all adolescent patients should be screened using the NICE guidelines. However, further analysis of risk factors and morbidity will help develop tailored recommendations for this specific patient population.

Background. Venous thrombo-embolism (VTE) amongst the hospitalised patients is a serious and prevalent problem jeopardizing patient safety. Most consensus based guidelines on this subject highlight the need for recognising and identifying the individuals at risk. The process of risk assessment allows the health care providers to take appropriate preventive measures e.g. administration of prophylactic doses of Enoxaparin. The risk assessment process itself depends on awareness amongst the health providers about this issue. This is best achieved by putting in place a system of alerts, reminders and mandatory intervention. Aim. Over the last 3 years, we developed and implemented a computer based electronic model of mandatory assessment for the risk of VTE in all adult patients admitted to our hospital. Methods. Using the fully integrated Electronic Patient Record system, a computer program was developed to introduce VTE risk assessment as a step in the admission process. This program also provided electronic reminders to the health care staff to perform the VTE risk assessment and prescribe thrombo-prophylaxis. As an electronic tool it makes auditing easy and accurate. To further enhance the clinical leadership on this matter compliance. We have now analysed our VTE risk assessment and thrombo-prophylaxis data for the last 33 months and our results show evidence that electronic models of VTE risk assessment is practically possible and effective. Results. Before the introduction of our VTE risk assessment tool, there was no formal process of risk assessment. The tool raised the profile of VTE risk assessment throughout the hospital and there was a rapid and significant increase in the number of patients having formal VTE risk assessment. In the last 3 months we have achieved a hospital wide average of 95.6% of adult in-patients being assessed for the risk of VTE. Prior to the implementation of risk assessment, the only measure of VTE prevention was prescribing rates for Enoxaparin prophylaxis. Our data shows that before the implementation of the tool, an average of 26% of all adult in-patients received thrombo-prophylaxis. The use of this tool has improved the thrombo-prophylaxis by about 10%. Nevertheless, the data shows that there is a gap between the number of patients assessed and those prescribed thrombo-prophylaxis. In the last three months we achieved VTE risk assessment in over 95% of patients admitted, demonstrating 51.6% at risk of VTE, but only 35% received Enoxaparin. Summary/Conclusions. Risk assessment for VTE is an essential step for reducing hospital acquired thrombosis and enhancing patient safety. Our data shows that an electronic model of VTE risk assessment is easy to implement and successful in achieving high rates of assessment. However, the prescription of thrombo-prophylaxis lags behind and this needs to be studied further. In the recent years, the focus has been on the need for accurate risk assessment. Now that computer based systems make that achievable, is it time to shift our focus to practical implementation of thrombo-prophylaxis?
THE CLINICAL SIGNIFICANCE OF JAK2V617F MUTATION FOR PHILADELPHIA-NEGATIVE CHRONIC MYELOPROLIFERATIVE DISEASES IN PATIENTS WITH SPLANCHNIC VEIN THROMBOSIS

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Background. Polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (IMF), collectively known as Philadelphia-negative (Ph-negative) chronic myeloproliferative diseases (MPDs) represent commonest causes of splanchnic vein thrombosis (SVT), including Budd-Chiari syndrome (BCS) and portal vein thrombosis (PVT). JAK2V617F mutation has been demonstrated in most Ph-negative chronic MPDs. High prevalence of JAK2V617F mutation in SVT reported in recent studies raised suspicion of an underlying MPD. Aims. We assessed the diagnostic value of JAK2V617F mutation in SVT in 68 patients with SVT (42 PVT, 19 BCS, 7 combined PVT and BCS) under follow up at outpatients clinics of Hematology and Gastroenterohepatology Departments of Istanbul Faculty of Medicine between years 2007 and 2010. Methods. Patients with SVT presenting with portal hypertension with/without cirrhosis were enrolled. Screening was performed for prothrombotic states, including genotyping for factor V Leiden, factor II, protein C, protein S, and antithrombin deficiencies, homocysteinemia and antiphospholipid antibodies. JAK2V617F mutation was detected by fluorescent resonance energy transfer probes and LightCycler techniques. Genotype assessment was based on melting curve analysis. Results. Patient features with respect to JAK2V617F mutation status are demonstrated in Table 1. JAK2V617F mutation was present in 42.1% of patients with BCS, 38.1% of PVT and 71.4% of combined PVT and BCS. Thirteen of 15 (86.6%) with overt MPD and 16 of 53 (30.1%) without overt MPD (patients with either normal blood counts or cytopenias), including 6 of 16 with BCS (37.5%), 7 of 33 with PVT (21.2%) and 3 of 4 with combined BCS and PVT (75%) possessed JAK2V617F mutation. JAK2V617F was associated with significantly higher platelet and leukocyte counts (Table 1). Most patients with JAK2V617F mutation had peripheral blood cell counts within normal range except for higher mean values for leukocytes (10525/mm3 (SD 6547)). There was a trend for higher LDH levels in JAK2V617F mutation carriers than in noncarriers (mean 523 U/L (SD 229.1), mean 481 U/L (SD 184.5), respectively; p=0.08). There was a significant positive correlation between JAK2V617F mutation status and leukocyte and platelet counts (r=0.445 and r=0.384, respectively). Receiver Operating Characteristic (ROC) curve analysis determined a platelet count of 199000/mm3 (area under curve; AUC=0.724, p=0.002) and a leukocyte count of 8150/mm3 (AUC=0.76, p=0.001) as best cut-off values for highest sensitivity and specificity ratios of JAK2V617F mutation in SVT. No relationship was observed between prothrombotic risk factors, JAK2V617F status, sites of thrombosis (PVT or BCS), presence of combined thrombosis and Hb, Htc, platelet and leukocyte counts. Conclusions. Signs of myeloproliferation may not be evident on peripheral blood if SVT is accompanied by portal hypertension and a hypersplenlic state. In our study, despite absence of overt signs of MPH, a substantial proportion of patients with SVT were shown to carry the JAK2V617F mutation. We found no relationship between prothrombotic risk factors and JAK2V617F status, site of thrombosis and presence of combined thrombosis. Our experience confirms that simple and rapid JAK2V617F testing on peripheral blood represents a key element of diagnosis of latent MPD in SVT.

Table 1. Patient features regarding JAK2V617F status in SVT.

JAK2V617F mutation was present in 42.1% of patients with BCS, 38.1% of PVT and 71.4% of combined PVT and BCS. Thirteen of 15 (86.6%) with overt MPD and 16 of 53 (30.1%) without overt MPD (patients with either normal blood counts or cytopenias), including 6 of 16 with BCS (37.5%), 7 of 33 with PVT (21.2%) and 3 of 4 with combined BCS and PVT (75%) possessed JAK2V617F mutation. JAK2V617F was associated with significantly higher platelet and leukocyte counts (Table 1). Most patients with JAK2V617F mutation had peripheral blood cell counts within normal range except for higher mean values for leukocytes (10525/mm3 (SD 6547)). There was a trend for higher LDH levels in JAK2V617F mutation carriers than in noncarriers (mean 523 U/L (SD 229.1), mean 481 U/L (SD 184.5), respectively; p=0.08). There was a significant positive correlation between JAK2V617F mutation status and leukocyte and platelet counts (r=0.445 and r=0.384, respectively). Receiver Operating Characteristic (ROC) curve analysis determined a platelet count of 199000/mm3 (area under curve; AUC=0.724, p=0.002) and a leukocyte count of 8150/mm3 (AUC=0.76, p=0.001) as best cut-off values for highest sensitivity and specificity ratios of JAK2V617F mutation in SVT. No relationship was observed between prothrombotic risk factors, JAK2V617F status, sites of thrombosis (PVT or BCS), presence of combined thrombosis and Hb, Htc, platelet and leukocyte counts. Conclusions. Signs of myeloproliferation may not be evident on peripheral blood if SVT is accompanied by portal hypertension and a hypersplenlic state. In our study, despite absence of overt signs of MPH, a substantial proportion of patients with SVT were shown to carry the JAK2V617F mutation. We found no relationship between prothrombotic risk factors and JAK2V617F status, site of thrombosis and presence of combined thrombosis. Our experience confirms that simple and rapid JAK2V617F testing on peripheral blood represents a key element of diagnosis of latent MPD in SVT.
Background. Deep venous thrombosis (DVT) is much less common in the upper than in lower extremity. Furthermore, there is limited information on risk factors for and the prognosis of upper extremity (UE) DVT in general population. Our aim was to study the contribution of thrombophilic markers, and in particular, factor VIII levels on risk factors for and the prognosis of UE DVT.

Methods. We carried out a retrospective observational study including 220 patients from our healthcare area eligible for evaluation. Both demographic and clinical features were recorded; these included age, sex, number of thrombotic episodes, type of event, and number of patients who suffered subsequent thrombotic recurrence. Factor VIII measurement was performed in citrated plasma by coagulometric method using factor VIII deficient plasma from Stago (STA Factor VIII, Diagnostica Stago, Asniéres sur Seine, France) and an STA R. coagulometer (Diagnostica Stago). Factor VIII levels above 150 IU/dl were considered as elevated (normal range 55-150 IU/dl). Results. Median age of patients enrolled at diagnosis of the first thrombotic event was 45 years (range 9-80). Eighty three of them (55%) were men and 68 (45%) women. Median number of thrombotic episodes was 1 (range 1-4). The first episode of VTE was a distal deep vein thrombosis (DVT) in 89 cases (54%). A pulmonary embolism in 34 (22.5%), a cerebral venous thrombosis in 7 (4.6%) and a mesenteric venous thrombosis in 4 (2.6%). Median factor VIII levels of the whole series of patients was 147.5 IU/dl (range 11-300 IU/dl). Out of 151 patients eligible for FVIII measurement 45 (29.8%) suffered through 72 episodes of VTE. Twenty seven out of 69 (39.1%) subjects who showed initially elevated FVIII levels (>150 IU/dl) presented with recurrent VTE versus 18 out of 82 (21.9%) of those whose FVIII plasma concentrations were within the normal range (p=0.021). Summary/Conclusions. Our findings confirm the statistically significant association existing between elevated FVIII plasma levels and thrombotic recurrence in patients with a previous episode of VTE. Inclusion of FVIII measurement in the routine thrombophilia screening is thus mandatory in order to identify this subset of patients. Whether such individuals are candidates to long term anticoagulant treatment on the basis of this higher risk of VTE recurrence remains unclear and future studies addressing this issue are warranted. email address: anagodoy1006@hotmail.com
study suggests that p-SLE children should be tested routinely for APLA, as this would identify patients with an increased risk for thrombotic manifestations. email: shanonaseem@yahoo.co.in

0183
IMPROVING ADHERENCE TO VTE (VENOUS THROMBOEMBOLISM) PROPHYLAXIS
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VTE (venous thromboembolism) is estimated to cause 60,000 deaths per annum in the UK. Hospital in-patients have many risk factors for VTE, such as acute illness, reduced mobility or surgery. As a result, NICE guidelines and government CQUIN targets aim to nationally reduce the morbidity and mortality associated with in-patient VTE through mechanical and pharmaceutical prophylaxis following focused risk assessment. This formed the initiative behind our study into improving education and awareness of VTE prophylaxis. Our aim was to assess and improve local trust adherence to VTE prophylaxis. Based in a major hospital in Central London, we set out to identify the barriers that needed to be addressed in order to improve compliance, and to implement changes that would raise awareness and reinforce the importance of VTE prophylaxis. By auditing inpatient populations on two separate occasions we were able to assess changes in compliance over a period of 6 months. Initially we ran a baseline audit to assess compliance prior to any awareness programme. Between audits, we ran a trust wide education programme: the introduction of a VTE risk assessment tool, setting up a VTE risk assessment proforma and specialised drug charts, and creating new hospital trust wide education programme: the introduction of a VTE risk assessment prior to any awareness programme. Between audits, we ran a concentrated education programme aimed at raising awareness and reinforcing the importance of VTE prophylaxis. Audit 1 (January 2010) n=250; Male to female ratio = 1.05:1; 64% aged greater than 60 years, 26% aged between 40-60 years; 11% with no risk factors, 35% with one risk factor and 54% with two or more risk factors. 18% of patients contraindicated for pharmaceutical prophylaxis. Audit 2 (July 2010) n=242; Male to female ratio = 0.9:1; 61% aged greater than 60 years, 23% aged between 40-60 years; 13% with no risk factors, 26% with one risk factor and 60% with two or more risk factors. 4% of patients contraindicated for pharmaceutical prophylaxis. Initially no standardised risk assessment on admission, 26% completed forms after education programme. Initial VTE prophylaxis prescribed for 49% of eligible patients, climbing to 74% after education programme. A concentrated education programme clearly improves compliance of VTE prophylaxis. However, it is a danger that without continued efforts, VTE prophylaxis and risk assessment can be overlooked. CQUIN targets of 90% are imposed on all NHS trusts for VTE prophylaxis and risk assessment, and it is likely some trusts will struggle without an awareness programme. Electronic patient records would increase the ability to not only audit VTE prophylaxis but also act as a reminder to risk assess accordingly for patients. Ideas for future development would be formal teaching at compulsory induction sessions for new employees and assigning a specialist nurse role in the trust.

0184
ANTITHROMBOTIC THERAPY IN NON-NEOPLASTIC CHRONIC PORTAL VENOUS THROMBOSIS IN CIRRHOSIS: RECANALIZATION AND LIVER FUNCTION EVALUATION
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1 H. Gregorio Marañón, Madrid, Spain
2 H.G.U. Gregorio Marañón, Madrid, Spain

Introduction. Non-neoplastic chronic portal vein thrombosis (PVT) is a frequent diagnosis in the course of liver cirrhosis, with reported prevalences of 0.6% to 15.8%. PVT can motivate life-threatening complications due to worsened portal hypertension, so anticoagulation therapy is challenging in these patients. OBJECTIVE: To analyze the response to antithrombotic therapy and changes in liver function tests in 28 patients with chronic PVT associated with cirrhosis. Patients and Methods. 28 consecutive patients with liver cirrhosis and chronic PVT were treated with antithrombotic therapy from 2004 to 2009. Hepatocellular carcinoma and known thrombophilic risks were ruled out. Therapy consisted in 15 days of therapeutic doses of low molecular weight heparin (LMWH) (enoxaparin) adjusted according to baseline coagulability (Table 1), followed by either prophylactic doses (40mg/day) of LMWH or acenocoumarol (target INR 2-3), during 6 months. Response was evaluated after 6 months.

Table 1

<table>
<thead>
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<th>Test</th>
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<th>INR</th>
<th>PT sec.</th>
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<td>19-20</td>
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If recanalization was complete, therapy was suspended. If recanalization was partial or no recanalization was observed, therapy was continued until response. RESULTS: From the 28 patients studied, 19 (68%) were males with a median age of 53 years (range 35-77). Cirrhosis was due to alcoholism (25%), virus (54%), mixed in 1 patient and other causes in 3 patients. PVT involved the portal trunk and/or branches in 19/28 (68%) patients, mesenteric vein in 2 patients and portal trunk and/or branches, mesenteric and/or splenic vein thrombosis coexisted in 7 patients. 19/28 (68%) of the patients had moderate or moderate-severe hypocoagulability range. Complete and partial thrombosis was seen in 18 and 10 patients at diagnosis, respectively.
Randomized comparison of the DAWN AC computer program and a simple manual nomogram for quality of warfarin dosing

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Background. In patients receiving warfarin, the quality of anticoagulant control as measured by the time-in-therapeutic range (TTR) for the International Normalized Ratio (INR) is a key determinant of risk for thromboembolic and bleeding events. Computer programs can assist in optimizing TTR, but experience in their utilization is limited. The purpose of this study was to determine whether a computer system (DAWN AC) was non-inferior to a two-step manual nomogram used in a hospital anticoagulation clinic, for quality of warfarin dosing. Methods. Patients receiving warfarin maintenance therapy with target INR range 2-3 in the anticoagulation clinic were randomized to management with the newly acquired DAWN AC computer system or the clinic’s standard of care, a simple manual dosing nomogram. After an initial run-in phase, study data collection started on February 1st 2010 and was completed on August 8th 2010. Primary outcome was the mean TTR calculated by an automated algorithm using the Rosendaal linear method. The non-inferiority margin was set at 4.5% lower TTR for DAWN AC compared with the nomogram. Approval from the Research Ethics Board of Hamilton Health Sciences was obtained and no patient informed consent was required. Results. Of the 1,298 patients initially randomized, 1,127 were still managed by the clinic after the run-in phase and entered the study (1,056 were randomized to the DAWN AC arm and 661 to the manual nomogram). The mean age of study patients was 69 ± 14 years and 62% were male. Main indications for anticoagulation were atrial fibrillation (48%) and prosthetic heart valves (25%). Mean follow-up was 172 days, encompassing 5,344 INR values and 155,041 patient days. Adherence to recommended warfarin doses was higher in the DAWN AC than in the nomogram group (99 vs. 90%; p<0.0001), and the average interval between consecutive INR measurements was similar in the two groups (21 ± 12 vs. 21 ± 13 days; p=0.1987). The primary analysis, mean TTR in the DAWN AC group was non-inferior to mean TTR in the nomogram group (71.0% ± 25.3 vs. 71.9% ± 22.9; non-inferiority p=0.0052). The effect of DAWN AC vs. nomogram on TTR was compared among subgroups for age, gender, warfarin pill size and primary indication and no significant interactions were found. Conclusions. Among patients receiving warfarin maintenance therapy with a target INR of 2-3 in an anticoagulation clinic, quality of anticoagulant control with the DAWN AC computer program was non-inferior to a simple two-step manual dosing nomogram. The nomogram could be a useful dosing tool for physicians without access to a computerized warfarin dosing system.

Motor disability in patients on oral anticoagulant therapy – a study on preliminary results from an Italian epidemiological study

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Background. OAT is chronically administered to prevent thrombotic events in a broad group of diseases having a narrow therapeutic range to balance the risk of hemorrhage and thrombosis, thus requiring a close monitoring of prothrombin time (PT) and the international normalized ratio (INR). Although PT/INR can be tested by a healthcare professional or by the patients (pts) themselves, the majority of pts are still managed by the clinic after the run-in phase and entered the study on February 1st; 564 were managed with DAWN AC, and 563 with the manual nomogram. The purpose of this study was to determine whether a computer system (DAWN AC) was non-inferior to a two-step manual nomogram used in a hospital anticoagulation clinic, for quality of warfarin dosing. Methods. Patients receiving warfarin maintenance therapy with target INR range 2-3 in the anticoagulation clinic were randomized to management with the newly acquired DAWN AC computer system or the clinic’s standard of care, a simple manual dosing nomogram. After an initial run-in phase, study data collection started on February 1st 2010 and was completed on August 8th 2010. Primary outcome was the mean TTR calculated by an automated algorithm using the Rosendaal linear method. The non-inferiority margin was set at 4.5% lower TTR for DAWN AC compared with the nomogram. Approval from the Research Ethics Board of Hamilton Health Sciences was obtained and no patient informed consent was required. Results. Of the 1,298 patients initially randomized, 1,127 were still managed by the clinic after the run-in phase and entered the study (1,056 were randomized to the DAWN AC arm and 661 to the manual nomogram). The mean age of study patients was 69 ± 14 years and 62% were male. Main indications for anticoagulation were atrial fibrillation (48%) and prosthetic heart valves (25%). Mean follow-up was 172 days, encompassing 5,344 INR values and 155,041 patient days. Adherence to recommended warfarin doses was higher in the DAWN AC than in the nomogram group (99 vs. 90%; p<0.0001), and the average interval between consecutive INR measurements was similar in the two groups (21 ± 12 vs. 21 ± 13 days; p=0.1987). The primary analysis, mean TTR in the DAWN AC group was non-inferior to mean TTR in the nomogram group (71.0% ± 25.3 vs. 71.9% ± 22.9; non-inferiority p=0.0052). The effect of DAWN AC vs. nomogram on TTR was compared among subgroups for age, gender, warfarin pill size and primary indication and no significant interactions were found. Conclusions. Among patients receiving warfarin maintenance therapy with a target INR of 2-3 in an anticoagulation clinic, quality of anticoagulant control with the DAWN AC computer program was non-inferior to a simple two-step manual dosing nomogram. The nomogram could be a useful dosing tool for physicians without access to a computerized warfarin dosing system.
ARE JAK2 MUTATION AND PAI -1 POLYMORPHISM RISK FACTORS FOR VASCULAR EVENTS IN ET PATIENTS?

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Background. The treatment of ET patients focuses mainly on lowering the risk and incidence of thrombotic and haemorrhagic complications. The tyrosine kinase activating JAK2 V617F point mutation has been found in 50% of Essential Thrombocytopenia (ET) patients and the relationship between the occurrence of this mutation and thrombotic events has been widely suggested. Additionally, it has recently been found that a 4G/5G polymorphism located in the promotor region of plasminogen activator inhibitor -1 (PAI-1) can also be a risk factor for deep vein thrombosis, a complication often observed in ET patients.

Aim. In this study we tried to identify the most important risk factors for vascular events based on evaluation of pathogenetic and prothrombotic mutations as well as hemostatic risk factors. Methods. We have enrolled 106 ET patients with ET (80 males and 26 females; mean age 54(23-82)). The control group (CG) consisted of 20 healthy persons: 6 males and 14 females (mean age 41(31-54)). We searched for JAK2 V617F, Factor V Leiden, C20210A prothrombin gene, MTHFR C677T gene mutations and PAI-1 polymorphism. We also evaluated plasma levels of: factors I, VIII, XII, AT, protein C and S and serum levels of: homocysteine, folic acid, vitamin B12. Results. The JAK2 V617F point mutation was detected in 48 (45.2%) ET patients. In ET patients with JAK2 point mutation compared to ET patients without JAK2 mutation, the higher levels of: red blood cells (RBC), white blood cells (WBC), hemoglobin (HGB) and hematocrit (HCT) were found. The results were as follows: RBC: median 4.77 T/l; P25-75%, 4.46-5.26 and 4.22T/l,3.8-4.52, p<0.001, WBC: median 8.5G/l, P25-75%, 6.8-10.60umol/l, <0.05. What is more, the folic acid level was 13.0g%,11.8-3.9, p<0.001, HCT: median 41.5%, P25-75%, 38.7-45.6 and 37.5%,34.0-40.2, p<0.001 respectively. JAK2 positive patients had lower CRP and free protein S levels compared to JAK2 negative patients (CRP: median 1.5 P25-75%,0.7-2.1 and 2.0, 1.75-2.45mg/l, p<0.05). In 21(19.8%) patients from ET group 37 thrombotic complications occurred and in 22(20.75%)ET patients bleeding episodes were noticed. 14(52.56%) patients with thrombotic complications were JAK2 positive, while only 5(11.65%) patients with bleeding episodes were JAK2 positive. The difference between these group was statistically significant (p<0.05). In JAK2 positive patients thrombotic episodes were found more often, while JAK2 negative patients were more prone to bleeding complications. The hyperhomocysteinemia was detected in 41(39.62%) of ET patients. The median homocysteine level was higher in ET patients compared to CG (11.10, P25-75%,8.74-13.28and 9.21,7.94-10.60umol/l, <0.05). What is more, the folic acid level was lower in ET patients compared to patients from CG (median 7.96,P25-75%,5.16-10.8 and 12.69,9.03.18.8 ng/ml, p<0.001). In ET group PAI-1 4G/4G polymorphism was found in 24(52.08%) persons while 4G/5G polymorphism was detected in 42 persons (39.62%). These mutations were present in the same proportions in patients with thrombotic and bleeding complications. Conclusions. The pathogenetic JAK2 point mutation seems to be also a thrombotic risk factor in ET patients. Higher RBC, HGB, WBC, HCT levels observed in JAK2 positive patients may aggravate the thrombotic risk. Hyperhomocysteinemia observed in ET patients may be due to lower folic acid level. PAI-1 polymorphism seems not to be a risk factor for thrombotic and bleeding complications.

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**Table 1. Thrombophilia factors in RPL cases.**

![Table 1. Thrombophilia factors in RPL cases.](image-url)
Background. Inherited thrombophilias are mutations with a Mendelian transmission that predispose carriers to a variably increased risk of venous or arterial thromboembolism. Commercially available testing kits include several such mutations, the clinical applicability of some being unproven. While the higher-risk mutations are associated with published risk-benefit analyses that clearly favour primary or secondary prophylaxis, other mutations confer a small increase in thrombotic risk that does not balance the increased bleeding risk of prophylaxis. Still other mutations carry a theoretically increased risk which has yet to be demonstrated in robust clinical series. The coexpression of more than one low-risk mutation facilitates the clinical decision to introduce prophylaxis, as the risk-benefit balance further shifts towards anticoagulation or antiaggregation; however, decisions become increasingly subjective as the number of low-risk or unproven-risk mutations increases, with it the perceived theoretical thromboembolic risk. Aims. To determine the frequency of hereditary thrombophilia mutation associations in a sample of patients, to characterize the clinical difficulties of deciding whether or not to institute prophylaxis. Methods. We reviewed all requests for molecular biology analysis of inherited thrombophilias, from patients with thromboembolic events, obstetric complications or familial thrombosis, received in our center between June 2005 (introduction of the strip-assay currently used) and January 2011, containing complete results for Factor V Leiden (FVL), Factor V HR2 Haplotype (FVHR2), Prothrombin G20210A, Beta-Fibrinogen 455 G>A, Plasminogen Activator Inhibitor 1 4G/5G and 5,10-Methylenetetrahydrofolate Reductase (MTHFR) C677T and A1298C, as well as levels of Antithrombin (AT) and Proteins C (PC) and S (PS). Results. We identified 2,048 individual patients fulfilling the inclusion criteria; 27 (1.3%) had no alterations in the parameters analyzed; 537 (26.2%) were high-risk patients with homozgyosity for FVL or deficiencies of AT, PC or PS (irrespective of other mutations), and 7 (0.3%) were heterozygous for both FVL and FVHR2 (irrespective of other mutations). Single heterozygous mutations were found in 142 (6.9%), while 72 (3.5%) carried single homozgyous mutations (excluding FVL), of whom 24 (2.4%) were homozgyous for a MTHFR mutation. Considering coexpression of low-risk mutations, 587 (28.7%) expressed two mutations, 469 (22.9%) expressed three, 182 (8.9%) expressed four, 23 (1.1%) expressed five and 2 (0.1%) expressed six. Conclusions. In this series we identified only 1.3% of patients with neither mutations in the strip-assay kit used, nor deficiencies of AT, PC and PS. A total of 26.5% presented with high-risk mutations which are well characterized in the literature, while 10.4% had low-risk mutations which are also well described. However, the majority of patients (51.6%) presented with two or three simultaneous low-risk mutations and a significant number (10.1%) with four or more mutations. This study highlights that fact that, though published series focus on individual mutations, the majority of patients have complex associations of low-risk mutations, the interaction of which is not well understood. Due to the number of mathematical combinations possible (over 1200), relevant cohorts analyzing the thrombotic risk of each are impossible to obtain. Current recommendations need to take into account this grey area, and further define guidelines for anticoagulation and antiaggregation.
Background. Molecular monitoring of BCR-ABL1 transcripts by real-time quantitative (RT-Q) is an integral part of the management of patients with chronic myeloid leukemia. Sequential BCR-ABL1 transcript results demonstrate the kinetics of response to tyrosine kinase inhibitors (TKI) therapy, condition for TKI progression free survival and can indicate impending loss of response. Inter-laboratory standardisation is necessary to provide consistent interpretation of patient results allowing therapeutic decisions and accurate comparison of clinical trial data. To date, standardisation to the International Scale (IS) has required sample exchanges with a national or international reference laboratory in order to establish and validate a methodology specific conversion factor (CF). Current methods for BCR-ABL1 RT-QPCR require time consuming preparatory, analysis and reporting steps. Automated and standardised BCR-ABL1 quantitation platforms would negate the requirement for a relatively labour-intensive, manual method and significantly improve turn around times enabling rapid (<2 hours) quantitative BCR-ABL1/ABL1 results with standard-
PDGFRB) in eosinophilia-associated myeloproliferative neoplasms (Eos-MPN) is characterized by the presence of multiple partner genes with heterogeneous breakpoints. However, accurate detection of these fusions is important for clinical management as they are associated with excellent response to imatinib mesylate. Aims. To develop quantitative real time PCR (RQ-PCR) assays to detect clinically significant overexpression of the 3’-region of PDGFRα and PDGFRB that may be indicated for fusion gene detection. Real-time PCR assays were designed for each gene; three assays 3’ to known breakpoints and three assays 5’ to known breakpoints. Where no fusion transcript is present, normal PDGFRα and PDGFRB expression (relative to the control gene) should be observed for all assays. However, when a fusion transcript is present, the three assays 5’ to the breakpoint region may detect overexpression compared to the three assays 3’ to the breakpoint region. Methods. Using the BioMark real-time PCR system and 48.48 Dynamic Array (Fluidigm) we tested pre-amplified cDNA from haematologically normal controls (n=16), samples from patients with unexplained eosinophilia that had previously tested negative for PDGFRα (n=12) or PDGFRB (n=3) fusions using conventional assays and samples with known PDGFRα (n=11) or PDGFRB (n=4) fusions. PDGFRα and PDGFRB expression was analysed using 6 RQ-PCR assays per gene and control gene assays for ABL1, BCR and GUSB. The 48.48 dynamic array allowed 2304 real time PCRs to be performed in one run enabling 48 samples to be analysed simultaneously in triplicates using 16 RQ-PCR assays. Results. Median Ct values were used to calculate the delta-delta Ct value for each assay and each sample using ABL1, BCR or GUSB as a reference. These data were then normalised to the expression level observed in the calibrator sample (normal human leukocytes). The difference between the median expression calculated using the assays 5’ and 3’ to known breakpoints was used to determine the relative overexpression of the 3’ end of PDGFRα or PDGFRB. The median relative overexpression was 44.3 fold (PDGFRα, p=0.001) for samples with PDGFRα fusions and 59.8 fold (PDGFRB, p=0.003) for samples with PDGFRB fusions compared with 0.66 fold (PDGFRα) and 0.06 fold (PDGFRB) in normal controls relative to ABL1. No significant overexpression of the 3’ end of the genes was found in the patients with unexplained eosinophilia when compared to normal controls (PDGFRα, p=0.186; PDGFRB, p=0.401). Statistically significant overexpression was observed in cases with known PDGFRB fusions compared to normal controls. The data were analysed using BCR and GUSB as reference genes. Conclusions. This simple, rapid and high throughput screen can be used to detect overexpression compared to the three assays 5’ to the breakpoint region. PDGFRα and PDGFRB fusions compared to normal when the data indicated the delta-delta Ct value for each assay and each sample using ABL1, BCR and GUSB as reference genes; three assays 3’ to known breakpoints and three assays 5’ to known breakpoints. Where no fusion transcript is present, normal PDGFRα and PDGFRB expression (relative to the control gene) should be observed for all assays. However, when a fusion transcript is present, the three assays 5’ to the breakpoint region may detect overexpression compared to the three assays 3’ to the breakpoint region. Methods. Using the BioMark real-time PCR system and 48.48 Dynamic Array (Fluidigm) we tested pre-amplified cDNA from haematologically normal controls (n=16), samples from patients with unexplained eosinophilia that had previously tested negative for PDGFRα (n=12) or PDGFRB (n=3) fusions using conventional assays and samples with known PDGFRα (n=11) or PDGFRB (n=4) fusions. PDGFRα and PDGFRB expression was analysed using 6 RQ-PCR assays per gene and control gene assays for ABL1, BCR and GUSB. The 48.48 dynamic array allowed 2304 real time PCRs to be performed in one run enabling 48 samples to be analysed simultaneously in triplicates using 16 RQ-PCR assays. Results. Median Ct values were used to calculate the delta-delta Ct value for each assay and each sample using ABL1, BCR and GUSB as a reference. These data were then normalised to the expression level observed in the calibrator sample (normal human leukocytes). The difference between the median expression calculated using the assays 5’ and 3’ to known breakpoints was used to determine the relative overexpression of the 3’ end of PDGFRα or PDGFRB. The median relative overexpression was 44.3 fold (PDGFRα, p=0.001) for samples with PDGFRα fusions and 59.8 fold (PDGFRB, p=0.003) for samples with PDGFRB fusions compared with 0.66 fold (PDGFRα) and 0.06 fold (PDGFRB) in normal controls relative to ABL1. No significant overexpression of the 3’ end of the genes was found in the patients with unexplained eosinophilia when compared to normal controls (PDGFRα, p=0.186; PDGFRB, p=0.401). Statistically significant overexpression was observed in cases with known PDGFRB fusions compared to normal controls. The data were analysed using BCR and GUSB as reference genes. Conclusions. This simple, rapid and high throughput screen can be used to detect clinically significant overexpression of PDGFRα and PDGFRB in Eos-MPN. The flexible nature of the dynamic array means that further assays could be designed to facilitate high throughput screening for other gene fusions in haematological malignancies.

0195

GENOMIC PROFILING USING SNP-BASED MICROARRAYS AS A DIAGNOSTIC TOOL IN ACUTE Lymphoblastic Leukemia

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Background. In acute lymphoblastic leukemia (ALL) specific chromosomal abnormalities provide important diagnostic and prognostic information. In recent years, it has become clear that ALL cells also frequently harbor relevant disease related submicroscopic chromosomal abnormalities (Kuiper et al. Leukemia 2007, 21, 1258-1266; Muliigian et al., Nature 2007, 446, 758-764). Although conventional karyotyping is generally considered as the gold standard in the genetic diagnosis of ALL, this method is limited by its low success rate due to an inadequate metaphase yield and/or a poor banding quality, and its inherent capacity to detect only those copy number changes that are large enough to be microscopically visible (5-10 Mb in size). Implementation of genome-wide high-resolution copy number profiling using genomic microarrays in routine diagnostics would allow the detection of clinically relevant abnormalities and, at the same time, would overcome the above mentioned limitation of karyotyping, since no culturing of the clinical samples is required. Aims. We explored whether microarray-based genomic profiling would be feasible as an alternative method to detect diagnostic and prognostic relevant genomic number aberrations (CNAs) in a routine clinical diagnostic setting. In addition, we aimed to develop a practical workflow for fast, objective and routine interpretation of CNAs obtained by microarray-based genomic profiling, thereby facilitating its application in a routine clinical diagnostic setting. Methods. We performed both conventional cytogenetic analysis and microarray-based genomic profiling, using the 250K Affymetrix® GeneChips arrays, of 60 ALL cases and subsequently compared conventional karyotypes with microarray-deduced copy number profiles. Results. Microarray-based genomic profiling resulted in a CNA detection rate of 95%, whereas for conventional karyotyping this was 61%. In addition, many small (< 5 Mb) genetic indications were not identified using the karyotypic platform. Many of these, sometimes focal, lesions harbor clinically relevant ALL-related genes such as CDKN2A/B, ETV6, PAX5 and IKZF1. Summary/Conclusions. We conclude that microarray-based genomic profiling serves as a robust tool in the genetic diagnosis of ALL. In addition, it outreaches conventional karyotyping in CNA detection, both in terms of sensitivity and specificity. Since balanced chromosomal abnormalities were not identified, it is recommended to apply FISH and/or other targeted methods for the detection of translocations with a known diagnostic and/or prognostic impact (e.g. t(9;22)/BCR-ABL1).

0196

PAX5 DELETION IS COMMON AND CONCURRENTLY OCCURS WITH CDKN2A DELETION IN B LINEAGE ACUTE LYMPHOBLASTIC LEUKEMIA

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Background and Aims. The PAX5 is essential in normal B-cell lymphopoiesis and deregulation of PAX5 function is believed to contribute to leukemogenesis in B-ALL. Although the common cytogenetic marker for minimal residual disease, early relapse or response to therapy. PAX5 apparent negative PAX5 immunohistochemistry suggests allele-specific regulation and haploinsufficiency of PAX5. Methods. We performed a comprehensive study using FISH, G-banding and IHC, to identify PAX5 deletion and immunoeexpression in 102 CD19+ clinical B-ALL cases (79 children and 33 adults) and investigated its relationship with common cytogenetic changes including BCR-ABL1, ETV6-RUNX1 and MLL rearrangements, and CDKN2A deletion. Results. The incidences of translocations and deletions were 2.5% and 10.0% in 33 children, and 0.0% and 18.2% in adults, respectively. The incidence of PAX5 deletion was higher than those of BCR-ABL1 (8.9%) or MLL rearrangements (5.1%) in children and than that of MLL rearrangement (3.1%) in adults. Most of patients with PAX5 deletion (83.3% of children and 100.0% of adults with PAX5 deletion) had concurrent CDKN2A deletion. PAX5 deletions were detected both in patients with positive and negative PAX5 immunoeexpression. Conclusions. In this study, we found that PAX5 is a common target in leukemogenesis of B-ALL along with CDKN2A. Owing to its frequent deletion in B-ALL, PAX5 could be used as one of the cytogenetic markers in diagnosis and monitoring of the disease. No correlation between immunoeexpression of PAX5 and deletion of PAX5 suggests allele-specific regulation and haploinsufficiency of PAX5. As a marker for minimal residual disease, early relapse or response to therapy, PAX5 appears to be more widely applicable than BCR-ABL1 or MLL rearrangement in B-ALL.

Figure 1. Incidence of PAX5 deletion, BCR-ABL1 rearrangement.
0197

SENSITIVE AND QUANTITATIVE DETECTION OF LOW LEVEL KIT D816V MUTATION IN PATIENTS WITH SYSTEMIC MASTOCYTOSIS USING A NANOFLOIDIC DIGITAL PCR ARRAY

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Background. Systemic mastocytosis (SM) is characterised by the abnormal proliferation and accumulation of clonally derived mast cells and is classified as a myeloproliferative neoplasm (MPN). The acquired activating point mutation 516V (D816V) of KIT can be detected in 80 - 95% of patients with adult onset SM. Because of its transforming activity, D816V is considered to play a primary role in SM development.

Methods. A mutation specific digital PCR assay was developed for sensitive detection and quantification of D816V using the Fluidigm Biomark real time PCR system and BioMark digital array. DNA samples from putative SM cases (n=24) and normal controls (n=12) were analysed in a blinded fashion for the presence of the D816V mutation and results were compared to those obtained using an allele-specific competitive blocker (ACB) PCR assay. Analysis of serial dilutions of the HMC-1 cell line (50% - 0.01% D816V) were tested to determine the limit of detection of the assay. Results. Testing of patient DNA by digital PCR showed 97% concordance with the ACB PCR results with the levels of quantification ranging from 0.01% - 37.9%. One case was found to be negative by ACB PCR but showed amplification in 3 reaction chambers in the digital PCR assay (mutational load of 0.01%). Amplification of D816V was not observed in normal control samples (n=12) or negative control panels demonstrating that the assay is highly specific for the mutated allele and that no amplification occurs from the wild type allele. Testing of HMC-1 cell line DNA serially diluted in DNA extracted from normal peripheral blood determined that the lowest limit of detection of the digital PCR assay was 0.01% compared to 0.1% for the ACB PCR. Conclusion. The nanofluidic digital PCR array used in conjunction with a novel mutation specific real time PCR assay allowed sensitive detection and quantification of KIT D816V to 0.01% in genomic DNA samples. The use of the digital array enables enhanced detection of rare mutated alleles in a high background of wild type DNA as the sample is partitioned prior to amplification. The detection sensitivity of 0.01% is greater than that achieved using any other methodology published to date. This enhanced sensitivity may increase the detection of D816V in samples with low mast cell content helping to improve the diagnosis and clinical management of patients with SM.

0198

THE FREQUENCY AND IMPLICATION OF GENE MUTATIONS INVOLVING RECEPTOR TYROSINE KINASES AND RAS PATHWAYS IN CHILDHOOD ACUTE MYELOID LEUKEMIA WITH FOUR MAJOR RECURRENT GENETIC ABNORMALITIES

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Background. Two-hit model of leukemogenesis has been proposed for acute myeloid leukemia (AML). Recurrent chromosomal translocations of t(8;21), inv(16), t(15;17), and MLL rearrangements are considered class II mutations. The implication of class I mutations in childhood AML with the 4 major recurrent genetic abnormalities is not clear. Aims. We sought to determine the frequencies of class I mutations involving receptor tyrosine kinases (RTKs)/JAK2/RAS signaling pathways in childhood AML with the 4 major recurrent genetic abnormalities and to assess their prognostic impact. Methods. Two hundred and one consecutively diagnosed patients with childhood AML were diagnosed between 1996 and 2010. Ninety-nine patients had the 4 major recurrent genetic abnormalities which were detected by cytogenetic analysis and/or RT-PCR. MLL gene rearrangement was first screened by cytogenetic, Southern blot or FISH analysis, followed by RT-PCR to detect common MLL fusion transcripts. Gene expression and DNA/RNA parallel PCR to identify the rare MLL partner genes. Class I gene mutations, including FLT3/ITD, FLT3/TKD, C-KIT, and C-FMS (RTKs); N-RAS, K-RAS, and PTPN11 (RAS pathway); and JAK2V617F, were examined. Mutational analyses were performed by DNA/RNA PCR followed by direct sequencing for genes of RTKs and RAS pathways, and allele-specific PCR for JAK2V617F. Fifty-seven patients treated with Taiwan Pediatric Oncology Group AML, APL and AML 97A (for non-AML) protocols were analyzed for the prognostic impact of gene mutations. Results. The frequencies of gene mutations in each genomic subtype of AML are shown in Table 1.

Table 1.

Mutations involving RTKs/JAK2/RAS pathways occurred in 51.3% of t(8;21)AML, 70.6% of inv(16), 62.5% of t(15;17) and 40.7% of MLL translocations. Taken together, 53.5% of patients had class I gene mutations; 38% for RTKs, 17% for RAS pathway and 3% for JAK2V617F, in the whole cohort of AML patients carrying 4 major recurrent genetic abnormalities; 5 of them had multiple gene mutations. The 5-year event free survival (EFS) of 57 patients was 72±6% (mean±SD), 92% for ITD, 92% for TKD, 90% for V617F (t(15;17), 78% for inv(16), 68% for t(8;21), and 67% for MLL rearrangement. Comparison of 5-year EFS or OS between gene mutation-positive and mutation-negative patients in each genetic subtype of AML showed that neither gene mutation of RTKs nor gene mutation of RAS pathway had a significant influence on outcomes. Conclusions. Our results showed that 53.5% of pediatric AML with the 4 major recurrent genetic abnormalities harbored mutated genes involving RTR/JAK2/RAS pathways but presence of gene mutations did not have influence on outcomes.

0199

MOLECULAR DETECTION OF CIRCULATING SEZARY CELLS IN PATIENTS WITH MYCOSIS FUNGOIDES: COULD IT PREDICT A FUTURE DEVELOPMENT OF SECONDARY SEZARY SYNDROME? A SINGLE MEDICAL CENTER EXPERIENCE

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Background. Mycosis fungoides (MF) is a primary cutaneous T-cell lymphoma and its prognosis heavily depends on clinical stage. While the majority of patients with early-stage disease have an excellent prognosis with life expectancy similar to normal population, few cases progress to secondary Sezary syndrome (sSS), which carries a dismal clinical outcome. An early detection of sSS is crucial for clinical decision making, but a reliable test is currently not available. Aims. To investigate the PCR detection of blood T-cell receptor gamma gene (TCRG) rearrangement in patients with MF and its role in predicting clinical outcome with a special focus on cases with B0-1 of blood staging. Method. After the institutional approval of the study, 135 cases of MF/SS were identified in our medical informatics system. The clinical staging, skin histology and circulating Sezary cell count, flow cytometric analysis of blood sample and PCR detection of TCRG rearrangement in skin and blood specimens were retrospectively analyzed. Kaplan-Meier survival analysis was performed to study the follow up evaluations and patients’ survivals. Results. Of 135 total cases, 74 (54.8%) are male and...
61 (45.2%) are female. The median age is 60 years with range of 21-93. Fifteen cases (11.1%) fulfilled the diagnosis of SS and 120 were MF. The initial clinical staging includes T1 in 79 (58.5%), T2 in 41 (30.4%), and T3/T4 in 15 (11.1%) cases. In addition, 7 (5.2%) cases were staged as N1-N3 or Nx. No one showed evidence of visceral organ involvement (M0) at initial presentation. At the initial evaluation of blood, the median of Sezary-like cells is 4% of total lymphocytes with range from 0% to 70%. By flow cytometry, the median of percent CD4+CD7- T-cells is 3.8 with range of 0-83, and the median for CD4+CD26- T-cells is 5.6 with range of 0-87. The median CD4:CD8 ratio is 2.1 with a range of 0.5-50. Of 131 cases with PCR/TCRG performed on blood, 44 (33.6%) are positive for clonal TCRG rearrangement and 87 (66.4%) are negative. When stratified by the diagnoses, the patients with MF showed a 26.5% (31/117) positive rate for blood T-cell clone, of which approximately 50% (10/20) had identical T-cell clone in skin. Follow up evaluation showed conversion into sSS in 50% (5/10) of the cases with positive blood T-cell clone (MF-1) (estimated mean interval = 41.8 months) in comparison to none in the cases without (MF-2) (0/31) (P<0.0001) (Figure 1A). Interestingly, 4 of 5 cases with sSS had an identical T-cell clone in skin, while the remaining case did not have the test performed on skin for clonal comparison. Kaplan-Meier survival analysis demonstrated a poor clinical outcome in the group with blood T-cell clone in comparison with the one without in overall survival (P<0.0001) (Figure 1B1) and progression-free survival (P<0.0001; HR = 22.6) (Figure 1B2). Conclusion. The findings suggest a role of molecular detection of blood T-cell clone in predicting sSS. Due to amplification of non-neoplastic T-cell expansion in significant cases, comparison of blood T-cell clone with skin may have a confirmatory value for defining the clonal nature.

Figure 1. Kaplan-Meier survival analyses.

0200
BCR-ABL QUANTITATION: TO IS OR NOT TO IS, THAT IS THE QUESTION?
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Reverse-transcription real-time quantitative polymerase chain reaction (RT-PCR) measurement of BCR-ABL oncogene expression is now a routine monitoring strategy for tyrosine kinase inhibitor (TKI) treated chronic myeloid leukemia. A major molecular response of at least a 3-log reduction in BCR-ABL expression is associated with favorable progression to disease-free survival. Data from our UK NEQAS LI BCR-ABL quantitation programme has shown that there is an average 105% inter laboratory CV (range 78%-130%). Recently, to overcome this problem, a new International Scale (IS) for BCR-ABL measurement has been proposed. The aim of this study was to determine whether the use of the IS significantly reduces the problem. The IS uses the log reduction in BCR-ABL expression as the measurement on an international reporting scale to allow consistent interpretation of individual patients response and comparison of response rates between clinical trials. Blood.2008;112:3330-3338.

0201
This abstract has been withdrawn.

0202
HIGH-RESOLUTION COPY NUMBER ARRAY IN THE MOLECULAR CYTOGENETIC DIAGNOSTICS OF PEDIATRIC MALIGNANT HEMATOLOGICAL DISORDERS
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Background and Aim. The importance of genetic characterization with cytogenetic and molecular genetics methods is well established in the diagnosis, prognosis prediction and determination of therapy in the management of malignant hematological disorders. The methods used today have limited detection sensitivity and resolution but are also relatively expensive. In order to validate the use of a genome-wide high resolution DNA copy number analysis in combination with conventional cytogenetic and molecular cytogenetic analysis, a high-density oligonucleotide based single nucleotide polymorphisms (SNP) array was performed on pediatric hematological malignancies. Material and methods. Bone marrow aspirates were consecutively collected from 56 children referred for diagnosis and treatment at The Queen Silvia Children’s Hospital during the years 2006 to 2009. All patients were diagnosed with malignant hematopoietic disorders, including 24 patients with precursor B lymphoblastic leukemia (B-ALL), 11 with T-cell acute lymphoblastic leukemia (T-ALL), 15 with acute myeloid leukemia (AML), two with myelodysplastic syndrome (MDS), two with chronic myeloid leukemia (CML), one with acute promyelocytic leukemia (APL) and one with Burkitt lymphoma (BL). Genetic alterations were investigated with molecular allelokaryotyping (Human Mapping 250K Nsp Array) in combination with conventional and spectral karyotyping (SKY), fluorescence in situ hybridization (FISH) analysis. Results. A total of nine balanced reciprocal translocations, 36 unbalanced translocations, 12 deletions and one inversion were detected by G-banding and/or SKY. FISH and RT-PCR confirmed all the known balanced translocations and the inversion. Eleven of the karyotypes in the material were complex, including four B-ALL, three T-ALL and four AML patients. The array analysis identified gains, losses and CNN-LOH in all the acute leukemia types, except for in the APL. The SNP information showed different patterns in the different leukemia entities. Gains dominated the B-ALL patients and losses the T-ALL and AML patients. Nearly all of the CNN-LOH was detected in the T-ALL patients. The SNP array was particularly helpful in detecting additional chromosome aberrations in the B- and T-ALL patients. The SNP array revealed the exact breakpoints in the unbalanced structural aberrations and small deletions were discovered. For example, loss of CDKN2A (p921) was detected in four T-ALL patients but the array revealed ten losses. Also, the array verifies unbalanced structural rearrangements as well as confirming the balanced. None of the balanced translocations were identified by the array and small pathological clones detected by conven-
tional cytogenetics and/or FISH were not detected as the proportion of these cells was too small. Conclusions. Here, we conclude that the addition of SNP array based karyotyping combined with conventional cytogenetics improve the genetic characterization of pediatric hematological malignancies. The two techniques enhance the cytogenetic image and should not be contrasted as they meet distinct important roles.

Since balanced translocation cannot be detected with the currently used SNP-arrays and tumor specific translocations are very important diagnostic and prognostic indicators we suggest that SNP-based array is a valuable adjuvant tool in the cytogenetic diagnostics of pediatric leukemias but cannot replace currently used techniques, i.e. G-banding and FISH.

0203

HSP 90 AS A POSSIBLE INDICATOR OF DISEASE DETERIORATION IN CHRONIC MYELOID LEUKEMIA

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Heat shock proteins 90 and 70 (HSP 90 and 70) are chaperones important for stability of various proteins. One of their client proteins is BCR-ABL kinase, fusion protein playing a crucial role in chronic myeloid leukemia (CML). The BCR-ABL/HSP90 complex stabilizes BCR-ABL kinase and decreases cell sensitivity to apoptosis (Shiotu Y. Blood 2000; 96:2284-2291, Wu, L.X. Leukemia 2008; 22:1402-1409). Moreover Src kinases and other proteins playing role in CML resistance to therapy are also clients of HSP90. The aim of our study was to find out whether the increasing levels of HSP90 and HSP70 might be associated with resistance to therapy and disease progression. We tested the expression levels of HSP90 and HSP70 in 41 CML patients with various responses to imatinib both on protein (western blots) and mRNA levels (real-time PCR). Eight more patients were examined during the course of therapy and development of resistance. Eight healthy donors and K562 cell lines were used as controls. By western blot analyses we found that the expression levels of Hsp70 did not markedly changed between different responses to therapy, between different disease stages or even between CML patients and healthy subjects. On the other hand, we found high variability in HSP90 protein levels. Patients at diagnosis and patients in good response to therapy (major molecular response) had low expression levels of HSP90 which were very similar to the HSP90 levels in healthy individuals. Patients in hematological relapse and in advanced CML phases (AP/BC) exhibited overexpression of HSP90. Very high HSP90 expression levels were also found in cell lines derived from CML blast crisis. Results indicate that HSP90 protein level well correlates with the disease status. In patients where the HSP90 protein expression was measured in course of disease, the increase of HSP90 level preceded relapse by 2 month at least. Thus the increasing HSP90 level seems to signalize arising resistance to therapy and disease progression. The amount of HSP90 also well correlates with the BCR-ABL transcript level in most cases. The only discordance was in samples from CML diagnosis, where BCR-ABL transcript level was high while HSP90 level was very low. This suggests different intensity of HSP90 action at CML diagnosis and blast crisis. In the same samples we also tested HSP90 expression on mRNA level by real-time RT-PCR. We quantified expression of two HSP90 isoforms - alpha and beta. The mRNA profiles showed high similarity with data obtained from western blot analyses. The analyses showed that increase in HSP90 levels associated with disease deterioration is probably mainly represented by HSP90beta isoform. These results suggest that HSP90 expression at protein as well as mRNA level may be an additional indicator of disease deterioration, a risk factor of poor therapy response. The advantage of such a non-specific general marker is in the possibility of monitoring the leukemic burden independently of the mechanism of developing resistance.

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0204

POLYPLOIDY DETECTION DURING CYTOGENETIC INVESTIGATION OF BONE MARROW FOR THE ASSESSMENT OF THE COURSE OF SOME HEMATOLOGICAL MALIGNANCIES

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Background. Polyploid metaphases are frequent findings during conventional cytogenetic investigation of bone marrow aspirate from patients with different hematological malignancies. However, they aren’t recognized in most cases as a pathological feature, referring polyploid metaphases to the signs of normal megakaryocytes presence in the specimen. Nevertheless, endomitotic events in megakaryocytes are rare phenomena in normal bone marrow, as well as spontaneous polyploidization of hemopoietic cells, and contribute approximately 1% of endomitotic events at the same time in short cultures. The aim of the Study was to evaluate the occurrence of polyploid metaphases in bone marrow aspirate from patients with myeloid malignancies. Materials and Methods. The cytogenetic study was carried out for bone marrow aspirates from 76 patients: 58 of them had chronic myelogeneous leukemia (CML), 2 had polycythemia vera, 1 had myelodysplastic syndromes (MDS). The standard G-banded method of cell cultivation was used. Karyotype analysis was performed using G-method of differential staining. Up to 25 metaphases were analysed from every aspirate. Results. Polyploid metaphases were observed in 14 (35.1%) patients with MDS, and appeared in all cases as a mosaic feature (4.2-50.0% of whole population of metaphases, in a given patient). Polyploidy as the only variation of karyotype was diagnosed in 6 patients; the other 8 had additional chromosome abnormalities. Polyploid cells were clonal in 3 (7.7%) patients with MDS, and constituted 12.5-50.0% of cytogenetically visible cell population of the specimen. The increased ploidy varied from 3n to 13n, but the most frequent (92.8%) were tetraploids in different proportions. Polyploid metaphases (tetraploids alone or in combination with triploids in 1 patient) were detected in 7 (20.0%) of CML patients and constituted 5.0-40.0% of all metaphases. Polyploids appeared after the beginning of treatment in 2 patients with CML, and were the sole change in the karyotypes, while the Ph-chromosome was already cytogenetically undetectable. Both 2 patients with PM had abnormal polyploid metaphases: 2 neartetraploids in one patient and 2 variants of neartetraploids in another. Conclusions. The primary mutations in myeloid malignancies occur in multipotent cells-progenitors, which mean that such mutations could cause effect on both myeloid, erythroid and megakaryocytic lines. So, the explanation of an increased ploidy in the considerable amount of bone marrow cells may be a presence of pathologically changed megakaryocytes with increased endomitotic activity, which indirectly depict retention of residual malignant clone, even if proliferating blasts are eliminated. The other possibility is that discovered polyploids, especially in patients with blast crisis are blasts. Moreover, reports have shown that polyploid cells have decreased availability to proliferate. This could result in a small amount of polyploid blast cells during cytogenetic investigation. So, polyploid metaphases should be carefully detected, even if discovered in only one copy per sample, because such findings may show the presence of the residude pathological clone with low proliferative index, availability of several copies of oncogenes and enhanced heterochromatization, which could cause together higher tolerance for injurious effects of antineoplastic agents.

0205

MUTATIONS OF NPM1, FLT3-ITD/TKD AND CEBPA IS VERY RARE IN MYELOID SARCOMA - FROM AN EVALUATION OF THE DIAGNOSTIC UTILITY OF CYTOGENETIC AND MOLECULAR GENETIC STUDIES IN ISOLATED MYELOID SARCOMA

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‡Background. Myeloid sarcoma (MS), an AML equivalent, refers to one or more tumor masses consisting of myeloid blasts occurring at an anatomical site other than the bone marrow (BM). MS is very rare consisting of 3-5% of AML, showing worse prognosis than other AMLs. Common chromosomal abnormalities in MS include ALL or ETV6-RUNX1 rearrangement, monosomy 7 and trisomy 8. Few studies have
studied the incidence of mutations of NPM1, FLT3 or CEBPA - the good prognosis factors of AML - in MS. The cytogenetic and the molecular genetic abnormalities were investigated using G-banding, FISH and mutation analysis. Aims. We identified the incidence of recurrent genetic changes of AML in MS and evaluated the diagnostic utility of cytogenetic and molecular genetic studies in detecting BM involvement of MS without morphological evidence. Methods. Fourteen cases (1.8%) of MS were selected based on pathological diagnosis out of 784 AML patients Seoul National University Hospital between Jan 1997 and Oct 2010. Serial specimens of primary tissue of MS and BM at diagnosis and in disease monitoring were included in the study. Interphase FISH technique was used to detect ETV6-RUNX1, CBFB-MYH11, PML-RARA, MLL, or BCR-ABL rearrangements on tissue and BM specimens. G-banding was performed on the BM specimen. Mutation studies including NPM1, FLT3-ITD/TKD and CEBPA mutation were also performed on tissue and BM specimens. Results. The incidence of MS was 1.8% (14/784, 5 males and 9 females, median age 51.1 years ranging from 1 month to 67 years). Most of them (85.7%, 12/14 patients) were presented as isolated MS without BM involvement at initial diagnosis. Affected sites include gingiva, neck lymph node, small bowel, abdominal wall, mediastinal lymph node, retro-molar area, palate, uterus, supraclavicular lymph node, and buttock. All 14 patients (1.8%) of MS were selected based on pathological diagnosis out of 784 AML patients Seoul National University Hospital between Jan 1997 and Oct 2010. Serial specimens of primary tissue of MS and BM at diagnosis and in disease monitoring were included in the study. Interphase FISH technique was used to detect ETV6-RUNX1, CBFB-MYH11, PML-RARA, MLL, or BCR-ABL rearrangements on tissue and BM specimens. G-banding was performed on the BM specimen. Mutation studies including NPM1, FLT3-ITD/TKD and CEBPA mutation were also performed on tissue and BM specimens. Results. The incidence of MS was 1.8% (14/784, 5 males and 9 females, median age 51.1 years ranging from 1 month to 67 years). Most of them (85.7%, 12/14 patients) were presented as isolated MS without BM involvement at initial diagnosis. Affected sites include gingiva, neck lymph node, small bowel, abdominal wall, mediastinal lymph node, retro-molar area, palate, uterus, supraclavicular lymph node, and buttock.

Out of 12 with isolated MS, 6 patients developed BM involvement during the treatment (median interval, 5.5 months ranging from 2.7 to 14.0 months). Among 12 with isolated MS, 2 had MLL rearrangement, and one had CBFB-MYH11. The other 9 showed normal karyotype. In one patient with isolated MS, MLL rearrangement was observed in both the tissue of MS (abdominal wall, 91.0% of all nucleated cells in iFISH) and the BM section (5.0%) at the initial diagnosis, without the morphological evidence of BM involvement. The morphological evidence of BM involvement developed in 4 months with additional gain of 1q. Patients who developed BM involvement of GS during treatment showed MLL or CBF-MYH11 rearrangement in both tissue and BM (2 cases), complex translocations involving chromosome 5, 4, 12, 15, 17 and 21, and loss of 7q; (2 cases). All 12 cases showed wild type of NPM1, FLT3-ITD/TKD and CEBPA genes. Conclusions. We confirmed that MLL and CBFB-MYH11 is relatively common cytogenetic abnormalities in MS. The mutations of NPM1, FLT3-ITD/TKD and CEBPA genes were not observed in MS in this study, which might be associated with the poor prognosis of MS than other AMLs. Our result suggests the potential diagnostic utility of cytogenetic/molecular genetic studies in detecting the occult BM involvement of MS without definite morphological evidence.

0206

MONITORING OF TOTAL WT1 EXPRESSION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA (CML): WT1 AS A HELPFUL MARKER BEIDES BCR-ABL

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WT1 encodes for a transcriptional regulator which behaves as an oncogene in leukemias. Although the mechanism of WT1 oncogenic behaviour has not yet been uncovered, WT1 expression is already used as a marker for monitoring minimal residual disease of patients with acute myeloid leukemia. Concerning CML, there is still very limited information about WT1 expression, most of the studies focused on WT1 expression significance for early prediction of relapses following allo-genic stem cell transplantation. Now, it is of interest whether monitoring of WT1 expression might be useful also for patients on tyrosin kinase inhibitors. In our study, we focused on WT1 expression in CML patients on imatinib. Altogether, we have examined 35 patients during the course of imatinib therapy: 4 patients exhibited optimal response, 8 suboptimal response and 23 therapy failure. Twenty % of those patients relapsed and the remaining patients stayed without relapse for more than 30 months (45,5 months in median). Real-time PCR was used according to Cilloni et al., 2004, B2M was applied as control gene. Predictive value of WT1 expression for development of haematological relapse was compared with that of BCR-ABL transcript and mutations in the BCR-ABL kinase domain. Before upcoming haematological relapse, WT1 expression was increased in about 60% of patients more expressively and in about 45% of patients even in median 2,3 months earlier as compared to BCR-ABL expression. As compared to BCR-ABL mutation analyses, WT1 increase predicted relapse earlier in about one third of patients. According to our experience, CML patients exhibiting suboptimal response and therapy failure represent a highly heterogenic group in terms of relapse emergence during the course of therapy. In our patients cohort, there were 70 % of suboptimal responders and 30 % of patients in therapy failure who did not relapse for more than 30 months of therapy despite of high levels of BCR-ABL (10 to 100%). Interestingly, exact WT1 levels even in the 12th month of therapy corresponded to relapses emergence during further course of the disease. Expression levels higher that 0,1 meant 90% probability of relapse. Taken together, our data indicate that WT1 might be a very useful marker for both relapses prediction during the course of therapy and patients stratification according to risk of relapse at the beginning of treatment. Monitoring of WT1 expression might be thus of advantage both for improving CML therapy outcomes and for further investigation of mechanism underlying different responses to therapy.

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Drug resistance and pharmacology

0207

PHARMACOGENOMIC PROFILE ASSOCIATED WITH HIGH SENSITIVITY AND LOW TOXICITY TO A COMBINATION OF GEMTUTUMAB OZOGAMICIN PLUS FLUDARABINE, CYTARABINE, IDARUBICIN IN CD33-POSITIVE ACUTE MYELOID LEUKEMIA

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Background. In acute myeloid leukemia (AML) the presence or absence of cytogenetic abnormalities allows the identification of favorable, intermediate and unfavorable subgroups. However, besides these specific subgroups, little is known about the genetic variations influencing specific drug-related phenotypes. Aims. To perform an exploratory pharmacogenomic study that relates genetic variations in multidrug enzymes and transporters genes with the efficacy and toxicity to treatment. Patients and Methods. We analyzed 94 CD33-positive AML patients younger than 65 previously untreated and enrolled in a phase III multicenter clinical trial combining low dose of Gemtuzumab-ozogamicin (GO) with FLAI regimen (Fludarabine, Cytarabine, Idarubicin) as Induction chemotherapy (eudact: 2007-005246-26; ClinicalTrials.gov NCT00909165). The induction regimen (GO-FLAI) included fludarabine (25 mg/sqm) and Ara-C (2 g/sqm) on days 1-5, idarubicin (10 mg/sqm) on days 1, 3, and 5 and GO (8 mg/sqm) on day 6. Hematopoietic stem cell transplant was planned for all high risk AML patients in first complete remission (CR) after consolidation with intermediate doses of Ara-C and idarubicin. Cytogenetics, multidrug-resistance phenotype, FLT3 and NPM mutation status, as well as WT1 quantitative expression analyses were performed at diagnosis in all patients. Furthermore, high-resolution single nucleotide polymorphism (SNP) array analysis (Affymetrix, Inc. Santa Clara, CA, USA) was also performed. The allelic frequencies of 1930 genetic variations of 225 absorption, distribution, metabolism and excretion were assessed using the new Affymetrix drug-metabolizing enzyme and transport (DMET Plus) genotyping platform (Affymetrix). All statistical analyses were performed using the R package 2.11.1. Results. Of the 94 patients, genotype results were evaluable for 91 cases. The median call rate was 99.48 (range, 96.32-100). Three samples were run in duplicate and results were “passed call rate”were compared across all the polymorphic sites, showing a repeatability of 99.99%. In an initial screening procedure, we tested the association among SNPs and response to the induction cycle (FLAI + Gemtuzumab-Ozogamicin). Therefore, the genotyping profile of 80 patients in complete (85%) and partial (3%) remission was compared to that of patients (12%) with no response. We found a highly significant difference (p < 0.001) in the allele frequency of 2 variants, in complete linkage disequilibrium, in the alcohol dehydrogenase enzyme (ADH1A). These variants were not associated with high risk AML, FLT3 and NPM1 mutations, but strongly influenced response to the induction phase also in a multivariate analysis. Since genetic polymorphisms may influence the toxicity of chemotherapy drugs, we stratified SNPs according to liver toxicity and a significant difference in the allele frequency of a member of the cytochrome P450 family which is involved in the alcohol metabolism (CYP2E1) was found to be associated with a grade I/II liver toxicity. Conclusions. A pharmacogenomic panel made up of 1 gene (ADH1A) associated with clinical outcome and 1 gene (CYP2E1) associated with toxicity was for the first time identified in AML patients younger than 65 years treated with a combination of GO and FLAI regimen.


0208

CELLULAR INHIBITOR OF PROTEIN PHOSPHATASE 2A DETERMINES BORTEZOMIB-INDUCED APOPTOSIS IN ACUTE LEUKEMIA CELLS

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Background. Bortezomib has excellent antitumor activity against multiple myeloma and mantle cell lymphoma via its proteasome inhibition. The multiple cellular targets affected by proteasome inhibition implicate the potential role of bortezomib in enhancing antitumor activities in other hematological malignancies, despite that currently bortezomib is only approved in mantle cell lymphoma and multiple myeloma. Our previous study has shown that down-regulation of phospho-Akt (p-Akt) plays a key role in determining the sensitivity of hepatocellular carcinoma cells to bortezomib-induced apoptosis. Aims. In this study we aimed to examine the antitumor activity of bortezomib and to further explore the mechanism by which bortezomib induces apoptosis in acute leukemia cells, particularly focusing on target(s) regulating p-Akt (such as protein phosphatase(s)). Methods. Several acute leukemia cell lines were used for in vitro studies. Apoptosis was examined by both flow cytometry and Western blot. Signal transduction pathways in cells were assessed by Western Blot. Gene silencing was done by small interference RNA (siRNA). Results. We demonstrated bortezomib differentially induced apoptosis in acute leukemia cells (Fig 1b and 1c). Importantly, bortezomib showed the similar induction of the proteasome activity in both sensitive and resistant cells, suggesting that bortezomib-induced apoptotic effect may be independent of its proteasome inhibitory effect (Fig 1d). Furthermore, we found that a novel oncoprotein, cancerous inhibitor of protein phosphatase 2A (CIP2A) in acute leukemia.

Figure 1. CIP2A in acute leukemia.
tein phosphatase 2A (CIP2A), a cellular inhibitor of protein phosphatase 2A (PP2A), mediated the apoptotic effect of bortezomib on acute leukemia cells. CIP2A expression is readily demonstrated in acute leukemic blasts (Fig. 1a). In accordance to our previous study on bortezomib, we showed bortezomib increases PP2A activity in sensitive acute leukemia cells (HL-60 and KG-1), but not in resistant ones (MOLT-3 and K562). We validated that bortezomib’s down-regulation of CIP2A was associated with the presence of Mcl-1 overexpression in CD34+CD38- cells. Down-regulation of CIP2A by siRNA overcame the apoptotic resistance to bortezomib in MOLT-3 cells (Fig 1e and 1f). Importantly, bortezomib exerts its antitumor activity with a specific effect on CIP2A or Akt in HL-60 cells protected from bortezomib-induced apoptosis, and down-regulation of CIP2A by siRNA overcame the apoptotic resistance to bortezomib in MOLT-3 cells (Fig 1e and 1f). Furthermore, we indicated that CIP2A negatively regulates PP2A in CD34+CD38- cells (Fig. 1e and 1f). Our results suggest that CIP2A’s sensitivity in vivo antitumor activity in HL-60 xenotransfused tumors (in nude mice), but not in CIPA2 overexpressed HL-60 tumors. Interestingly, HL-60 cells with ectopic CIPA2 expression had increased cell proliferation and DNA synthesis, as well as a more rapid xenografted tumor growth. Summary. In conclusion, this study has identified CIPA2 as a major molecular determinant of bortezomib’s sensitivity on acute leukemia cells and that CIPA2 may play an important role in leukemia biology. It is also implicated that focusing on interaction of oncoprotein and phosphatase and kinases could be a novel anti-leukemia strategy.

0209

THE MULTI-KINASE INHIBITOR TG02 DOWNREGULATES MCL-1 IN AML CELLS AND PREFERENTIALLY TARGETS CD34+CD38-CD123+ CELLS FROM SAMPLES WITH AN INTERNAL TANDEM DUPLICATION OF FLT3

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Background. In clinical trials, FLT3 inhibitors are reported to kill circulating AML blasts, but the bone marrow is protected. We have previously reported that niche-like conditions (fibronectin and a cytokine cocktail), significantly induced the toxicity of the FLT3 inhibitor, AG1296 to AML cells. Moreover, the toxicity of AG1296 to the quiescent, stem-cell-enriched leukemic CD34+CD38-CD123+ subset was completely abolished under niche-like conditions. The novel multi-kinase inhibitor TG02 has selectivity against cell cycle and transcriptional CDK4/6, and as well as FLT3. TG02 has efficacy in vitro models and induces apoptosis in primary AML cells including CD34+CD38-CD123+ cells. We have now evaluated the impact of FLT3 internal tandem duplications (ITDs) on the in vitro toxicity of TG02 under niche-like conditions. FLT3 ITDs are associated with over-expression of the survival molecule Mcl-1, particularly in the leukemic stem cell compartment. We have therefore investigated the impact of TG02 on Mcl-1. Methods. The 48 hour toxicity of TG02 to primary AML blasts and to the MOLM13 cell line, which harbours a FLT3 internal tandem duplication (ITD), were studied by flow cytometric viable cell enumeration. Mcl-1, phosphorylated (active) STAT5 and phosphorylated RNA polymerase expression were measured by flow cytometry. Protein expression analysis was performed with a kit from R&D systems. Phospho-Mcl-1, phosphorylated (active) STAT5 and phosphorylated RNA polymerase expression had increased cell proliferation and DNA synthesis, as well as a more rapid xenografted tumor growth. Summary. In conclusion, this study has identified CIPA2 as a major molecular determinant of bortezomib’s sensitivity on acute leukemia cells and that CIPA2 may play an important role in leukemia biology. It is also implicated that focusing on interaction of oncoprotein and phosphatase and kinases could be a novel anti-leukemia strategy.

0211

REGULATION OF CYP3A4 AT NFSE IN HEMATOPOIETIC CELLS INVOLVES RNA AND EP1A

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Background. CYP3A4 is the most abundantly expressed hepatic cytochrome P450 and significantly contributes to drug metabolism, with substantial intra- and inter-individual variability. Its regulatory mechanisms are still enigmatic, with complex interactions in the 5'-flanking region. Among those involved is a functional element (NFSE) of the CYP3A4 promoter (termed CYP3A4*1B) is associated with a lower incidence of pediatric (Felix, PNAS, 1998) and adult (Rund, Leukemia, 2005) therapy-related AML. Previous studies by
others demonstrated binding of nuclear proteins at NSFE in hepatic cells and we have found such binding in hematopoietic cell lines and primary AML patient cells. NSFE regulation appears to be stress-responsive (heat shock) (David-Kalis, 2005). Aims. 1. To analyze expression of wild type and polymorphic NSFE sequences in hematopoietic cells. 2. To identify the proteins which differentially bind to the wild type and polymorphic sequences. 3. To determine if the protein is stress-regulated by stress (such as heat and chemotherapy), possibly via HSRE, an RNA molecule that activates stress response genes such as HSF-1. Methods. We constructed luciferase reporter plasmids (pGCL vector) driven by the CYP3A4 promoter, using polymorphic and wild type sequences. These plasmids were transfected into hematopoietic cell lines: K562 (CML blast crisis), CCRF (T cell ALL) and KG1a (myeloid leukemia). HepG2 (hepatoma) served as a control. In addition, electrophoretic mobility shift assays (EMSA) were performed using nuclear extracts from various cells using radiolabelled probes corresponding to the wild type and polymorphic sequences. A streptavidin-biotin system was used to isolate the proteins binding at NSFE. Results. CYP3A4 reporter gene studies demonstrated 20-30% lower activity in KG1a, CCRF and K562 using the polymorphic compared to the wild type sequence while HepG2 showed higher activity with the wild type. The results for HepG2 confirmed results reported by Rebbeck (2005). EMSA demonstrated NSFE binding using nuclear extracts from CCRF cells, decreased following heat shock. Deletions detected in resistant cells, the heat shock effect was stronger. The streptavidin-biotin system demonstrated that the DNA binding complex at NSFE was present in the same elution fractions containing HSF-1. Mass spectrometry demonstrated elongation factor 1a (EF1a). EF1a is known to be activated by an RNA molecule (Shamovsky 2006). Treatment with RNase enhanced binding to NSFE, suggesting that RNA interferes with binding of nuclear proteins to the CYP3A4 promoter. Addition of EF1a antibodies to nuclear extracts increased binding. NSFE complex formation was further enhanced when RNase and anti-EF1a were used together. The influence of RNase on the signal of the complex using the polymorphic NSFE probe was greater than its effect on NSFE wild type. Additional factor(s) may facilitate(s) assembly of the complex. The ALBαα database of transacting factors states high homology of the 5'11bp-NFSE-9bp 3' region with the consensus binding site of C/EBPb. C/EBPb itself was found involved indirectly, reducing the strength of the complex by binding to the C/EBPb element, found upstream to NSFE. Conclusions. Regulation of CYP3A4 at NSFE in hematopoietic cells appears to be stress-responsive, involving RNA as well as EF1a, not previously reported for other CYP genes.

0212 INTERACTION OF THE COMMON HOC1 IN MYELOGENOUS LEUKAEMIA

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Background. The human organic cation transporter 1 (hOCT1) is responsible for the uptake of imatinib into chronic myeloid leukaemia (CML) cells. Its expression level and functional activity are powerful predictors of the clinical response to imatinib (Wang et al., Clin. Pharm. Therapeut. 2008, White et al., Blood 2007). Several single nucleotide polymorphisms (SNPs) in hOCT1 affect its expression and transport activity, contributing to inter-individual variation in clinical response. SNPs in hOCT1, particularly M420del (c.1260del, allele frequency 18.5% in European-Americans), can affect the action and pharmacokinetics of metformin, an anti-diabetic drug and a well known hOCT1 substrate (Shu et al., Clin. Pharm. Therapeut. 2008). In addition, M408V is another common hOCT1 SNP with allele frequency approximately 59.8% in European-Americans. Aim. Firstly to analyze the expression of the common hOCT1 variants M420del and M408V in a CML cell line model and secondly to investigate the effect of these SNPs on the clinical response to imatinib treatment in a large cohort of newly diagnosed CML patients. Methods. KCL22 CML cell line was selected for hOCT1-transplantation due to their low basal hOCT1 expression. The specific SNPs were introduced to the pcDNA-hOCT1 plasmid by site-directed mutagenesis. Stably transformed cell lines with various combinations of M420del and M408V SNPs were generated. The uptake of 14C-radiolabelled imatinib into different cells was measured by scintillation counter. Genomic DNA samples were prepared from peripheral blood from 182 newly diagnosed chronic phase CML patients prior to imatinib therapy. Genotyping for the M420del and M408V SNPs was performed by pyrosequencing and Sequenom MALDI-TOF MassARRAY. Results. Cell lines carrying the hOCT1 M420del and M408V SNPs demonstrated a statistically significant decrease in the uptake of the wild type KCL22 cells with undeleted M420 and M408 (p=0.001). Conversely, KCL22 cells with undeleted M420 and M408 had an increased imatinib uptake compared with cells with undeleted M420 and M408 (p=0.05). In KCL22 cells with both M420del and M408, uptake did not differ from the wild type cells. These data imply that both M420del and M408V play a role in the uptake of imatinib in the hOCT1 transporter, whereby M420del decreases uptake and M408 increases it. In clinical samples from 182 cases of newly diagnosed CML patients treated with imatinib, patients who carried the M420del allele (n=53) had a greater probability of imatinib resistance requiring a change of therapy (p=0.034) and treatment failure (i.e. resistance + intolerance; p=0.024, Kaplan-Meier log-rank test) than patients with undeleted M420. Patients carrying both M420del and M408 alleles (n=45) had a greater probability of imatinib resistance requiring a change of treatment (p=0.025) and treatment failure (p=0.014). However, patients with both M420del and V408 had comparable times to treatment failure to patients with both undeleted M420 and M408V. Summary/Conclusions. This study provides evidence on the functional significance of both M420del and M408V polymorphisms in hOCT1. It also demonstrates the importance of SNP interactions in determining clinical outcome. These SNPs may provide a potential use for patient-specific therapy in newly diagnosed chronic phase CML.

0213 PREDICTION OF IMATINIB THERAPY RESPONSE: A ROLE OF INTRACELLULAR TRANSPORTERS HOC1-T AND ABCB1

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Background. The role of intracellular IMA transport in overall resistance and in treatment outcome prediction have been intensively studied since main influx (hOCT1-T) and efflux (ABCBI) transporter proteins have been described. Several studies have shown the predictive value of the pretreatment activity/mRNA expression of these transporters with subsequent therapy response have been reported to date. However, different cell populations from patients who received various degree of pretreatment were used for these analyses. Therefore, several biases in the results and their interpretation may arise. Aims. In this report we demonstrate that the composition of analyzed material at the time of testing (i.e. percentage of different cell types) has critical impact on the resultant activity/mRNA expression of these transporters, and therefore it can significantly affect the overall correlation and data interpretation. Methods. We analyzed the differences in the hOCT1-T and ABCB1 mRNA expression measured in peripheral blood (PB) leukocytes (LEU), polymorphonuclear cells (PMNC) and mononuclear cells (MNC) of PB LEU obtained from healthy volunteers and patients with de novo CML. Additionally, we analyzed the changes in hOCT1-T expression during the first six months of IMA therapy and the relationship between the percentage of individual cells that comprise the total LEU count and hOCT1-T and ABCB1 mRNA expression assessed from the total LEU. Finally, we investigated the predictive value of the pretreatment mRNA expression levels of hOCT1-T and ABCB1 in selected cell populations with regard to the therapy response at six and twelve months of IMA therapy. Results. The hOCT1 mRNA expression was significantly higher in PB PMNC compared to MNC. Expression in each analyzed group of cells was always significantly lower in IMA naive de novo CML patients compared to healthy volunteers. This difference disappeared after the initiation of IMA therapy, suggesting that CML tumor burden and the degree of pretreatment at the time of monitoring were both influencing factors. Moreover we found the statistically significant relationship between hOCT1-T mRNA expression and the percentage of immature myeloid cells as well as BCR/ABL transcript levels in PB (both as indirect markers of tumor burden). Considering ABCB1 expression, it was significantly higher in MNC compare to PMNC. Similarly to hOCT1-T, a
correlation with percentage of immature myeloid cell was obtained. All three results suggest that both ABCF1 and ABCB1 mRNA expression level used as a prognostic factor should always be assessed in relation to the cell type in which expression was measured. Finally, regarding the therapy response prediction, no statistically significant relationship between the pretreatment levels of hOCT-1 or ABCB1 mRNA expression in different cell populations and therapy response, have been observed. Conclusion. The observed cell type dependence, tumor burden dependence, and other pre-analytical and analytical biases found in recent literature lead to the conclusion that the desired stratification of CML patients into responders/non-responders prior to IMA therapy is hard to obtain and these parameters are not suitable for routine clinical practice.

0214 P-GLYCOPEPTIDE AND BREAST CANCER RESISTANCE PROTEIN IN ACUTE MYELOID LEUKAEMIA CELLS TREATED WITH THE AURORA-B KINASE INHIBITOR BARASERTIB-HQPA

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Background. Aurora kinases play an essential role in orchestrating chromosome alignment, segregation and cytokinesis during mitotic progression. Aurora-B is a preferentially over-expressed in a variety of human malignancies. Over-expression of the ABC drug transporter proteins P-glycoprotein (Pgp) and Breast cancer resistance protein (BCRP) is a major obstacle for chemotherapy in many tumour types with Pgp conferring particularly poor prognosis in acute myeloid leukaemia (AML). Barasertib-HQPA is a highly selective inhibitor of Aurora-B kinase that has shown tumouricidal activity against a range tumour cell lines including those of leukaemic AML origin. Aims. Using leukaemic cell lines and primary samples we aim to investigate the specificity of barasertib-HQPA with particular reference to their ABC transporter status. Methods. We analysed response in a panel of leukaemic cell lines and 37 primary AML samples by measuring phosphoHistone H3 (pHH3) expression, the biomarker for barasertib-HQPA activity. Results. In this study we report the creation of the cell line OCI-AML5/DNR, which over-expresses Pgp but not BCRP or multidrug resistance-associated protein (MRP), through prolonged treatment of OCI-AML5 cells with daunorubicin. We demonstrate that Pgp (OCI-AML5DNR and KG-1a) and BCRP (OCI-AML6.2) expressing AML cell lines are less sensitive to barasertib-hQPA induced pHH3 inhibition and subsequent loss of viability compared to transporter negative cell lines. We also show that barasertib-hQPA resistance in these cell lines can be reversed using known Pgp and BCRP inhibitors. We report that barasertib-hQPA is not an inhibitor of Pgp or BCRP, but by using 14C-barasertib-HQPA that it is effluxed by these transporters. We measured Pgp and BCRP expression in 37 primary AML samples. 9/37 (24.3%) were positive for Pgp and 9/35 (25.7%) were positive for BCRP with a significant correlation (r=0.808) seen for co-expression. By measuring pHH3, we demonstrate that Pgp sensitive cell lines expressed higher pHH3 than ABCB1 (R vs NR p=0.016; R vs BC p=0.027, respectively). Expression levels of barasertib-HQPA was further reduced in primary samples (both transporter negative and transporter positive). A significant positive correlation was shown to exist between SLCO2A1 (OCT1) and SLCO2A4 (r=0.74, Sign at 0.000), ABCB3 (r=0.6, Sign at 0.000), SLCO3A1 (r=0.68, Sign at 0.000), SLCO1A2 (r=0.53, Sign at 0.000), SLCO2A5 (r=0.48, Sign at 0.001), and ABCB1 (r=0.48, Sign at 0.001). Clinically, of the influx transporters, only SLCO2A4 appeared to be important (between statistically significant levels R vs NR p<0.050), while for the efflux transporters ABCB3 appeared to be more important than ABCB1 (R vs NR p=0.016; R vs BC p=0.027, respectively). Additionally, when patients’ SLCO2A1, ABCB1 and ABCB3 mRNA levels were compared it was apparent that patients with high ABCB3 expression were failing treatment irrespective of SLCO2A1 expression (HC vs NR R vs BC p<0.001). Our data clearly indicated the importance of the ABCB3 efflux transporter in imatinib treated CML patients and propose its potential use as a predictor marker of treatment failure. We are testing this idea within a larger clinical cohort as well as against the clinical response with other TKIs.

0216 MOLECULAR MECHANISMS OF Nilotinib RESISTANCE AND REVERSAL OF RESISTANCE IN CHRONIC MYELOID LEUKEMIA CELLS

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Background. Chronic myeloid leukemia (CML) is a disease of hematopoietic stem cells, arising from a reciprocal translocation between long arms of chromosomes 9 and 22, known as the Philadelphia chromosome. This translocation causes a juxtaposition of ABL and BCR genes, resulting in a constitutively activated BCR-ABL fusion gene. Nilotinib disrupts the enzyme activity and increases in mitochondrial membrane potential. This study aimed to develop nilotinib resistance by modulating bioactives phingolipids metabolism with using glyoxyl ceramide synthase (GCS) and sphingosine kinase-1 (SK-1) inhibitors. Methods. Human Antiproliferative eeffects of nilotinib on K562 and 50 nM nilotinib-resistant K562 (K562/NIL-50) cells were determined by XTT cell proliferation assay. Changes in caspase-3 enzyme activity and mitochondrial membrane potential were determined by caspase-3 colorimetric assay kit and JC-1 mitochondrial membrane potential detection kit, respectively. Expression levels of Bcr-Abl, apoptosis related genes (bcl-2, bcl-xl, bax, caspase-3), ceramide synthase genes (CerS1-6), GSS and SK-1, drug transporter genes (mdr1, mrp1, bcrp, lrp) and Beta-actin as an internal positive control were assayed by RT-PCR. Results. IC50 values of nilotinib were calculated as 42 and 385 nM for K562 and K562/NIL-50 cells, respectively indicating that K562/NIL-50 cells gain about 10-fold resistance to nilotinib as compared to parental K562 cells. Apoptotic mechanisms were repressed in K562/NIL-50 cells as determined by decrease in caspase-3 enzyme activity and increases in mitochondrial membrane potential. Expression levels of Bcr-Abl gene were upregulated in K562/NIL-50 cells as compared to parental sensitive cells. Nucleotide sequence analyses of ABL kinase gene revealed that there was rate the mutation in nilotinib binding site of BCR/ABL oncogene in resistant cells. There was also an increase in expression levels of MRP1 gene in resistant cells. Besides, apoptotic Bax and CerS1 genes were downregulated and...
antiapoptotic GCS and SK-1 genes were upregulated in K562/NIL-50 cells. Inhibition of GCS and SK-1 by chemical inhibitors sensitized K562/NIL-50 cells to nilotinib as determined by cell proliferation and apoptosis analyses. Summary/Conclusions. Determination of genetics mechanisms of cellular resistance in response to nilotinib is very important. In conclusion, we determined mechanisms involved in nilotinib-resistance in CML cells. Our results also demonstrated that targeting antiapoptotic GCS and SK-1 genes, besides inhibition of BCR-ABL by nilotinib, may be a good way of treatment of CML.

0217 PHARMACOGENETICS IN CHRONIC MYELOID LEUKEMIA TREATMENT: A TOOL TO PREDICT THERAPY OUTCOME TO IMATINIB TREATMENT?
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2University of Insubria, Varese, Italy

Background. Imatinib mesylate (IM) is a selective tyrosine kinase inhibitor, that has achieved successful treatment outcomes and improved the life quality of chronic myeloid leukemia (CML) patients. However, some of the patients fail to achieve optimal response, and a substantial proportion of patients develop resistance to IM. Several determinants were known to be associated with the pharmacokinetics of imatinib with respect to absorption, distribution, and metabolism, influencing the systemic level or intracellular concentration of imatinib which might affect response to therapy. IM is a substrate for the adenosine triphosphate binding cassette (ABC) transporters, ABCB1 and ABCG2, whereas the active uptake of IM into cells is mediated by the human organic cationic transporter-1 (OCT1; SLC22A1). Also, IM is metabolized through first-pass drug metabolism by the cytochrome P450 - CYP1A2, CYP2C9, CYP2D6, CYP3A4 and CYP3A5. Genetic variations affecting genes involved in IM transport and metabolisms, however, role of single nucleotide polymorphisms (SNPs) in predicting therapy outcome remains to be established. Aims. The aim of present work is to correlate response to imatinib therapy in CML patients with common SNPs, alone or in combinations, in genes involved in pharmacokinetics of these drugs. Methods. We evaluated imatinib efficacy as: (a) molecular response: complete (CMoR; defined as disappearance of detectable BCR/ABL fusion gene transcripts by quantitative PCR) / partial (pMoR; defined as disappearance of detectable BCR/ABL fusion gene transcripts) / absent (MoR) after treatment (b) cytogenetic response: complete (CCyR; 0% Ph+ cells in marrow by conventional cytogenetics) / partial (pCyR; 0-35% Ph+ cells in marrow) / absent (CyR) after 12 months treatment. We analyzed 10 candidate gene SNPs: in 3 genes associated with imatinib transport (ABCB1, ABCG2 involved in drug efflux and SLC22A1 involved in imatinib cell uptake) and 4 genes associated with drug metabolism (CYP1A2, CYP2C9, and CYP3A5). Genotyping was performed by Real Time PCR using TaqMan probe. ABCB1 phenotype was defined according to specific SNPs combinations as follow: Low Transporters (LT): patients carriers at least 3 polymorphic alleles; Intermediate Transporters (IT): patients carriers 2 polymorphic alleles and Extensive Transporters (ET): patients carriers no more than 1 polymorphic allele. Results. So far, 57 patients with CML treated with imatinib were enrolled: 11 (SM/ΦE; age 54±10 years achieved CMoR, 24 (14M/10F; age 54±17 years) pMoR and 22 (7M/15F; age 58±16) MoR. 51 patients (22M/29F; 55±15 years) showed CCyR and 6 (2M/4F; 56±16 years) pCyR. Cytogenetic response was not associated with genetic profile. However SNPs in ABCG2 (rs2231137), but not in ABCB1 and cytochrome, were significantly associated with molecular response. Moreover, 4 (37%) patients with CMoR had ABCB1 LT phenotype, while only 2 (9%) patients with MoR were LT, (CHI² test: P = 0.045). Conclusions. Preliminary results showed that the treatment outcomes, especially for molecular response, of imatinib therapy could be predicted using a novel, multiple candidate gene approach based on the pharmacogenetics of IM.

ASPIRIN RESISTANCE IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS
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Background. Low-dose aspirin (100mg/die) improves the survival of patients with cardiovascular events; however, about 25% of the patients treated with aspirin present thromboembolic complications (aspirin resistance). Pharmacological resistance is defined as the inability of aspirin to inhibit platelet aggregation and thromboxane production. Patients affected by myeloproliferative neoplasms (MPN) are at risk for thromboembolic complications and low-dose aspirin are effective also in these patients in reducing their thromboembolic risk. At present, few is known about aspirin resistance in this set of patients. Aims. This study explores pharmacological aspirin resistance in patients with MPN, in particular with polycythemia vera (PV) and essential thrombocytemia (ET), evaluated with platelet aggregation and serum thromboxane (TxB2) assay. Patients and Methods. We studied 123 MPN patients (41 PV and 82 ET), 83 were treated with aspirin (100mg/die) (MPN-ASA) and 40 were not (MPN-basal). PV and ET were diagnosed in agreement with WHO criteria. As controls we studied 50 patients treated with aspirin (100mg/die) for secondary prevention of thrombosis (Controls-ASA and Controls) and 42 healthy subjects (Controls). Platelet aggregation under 1 mM arachidonic acid (AA) stimulus was evaluated with Born’s method. Serum thromboxane B2 (TxB2) was measured with ELISA assay (Thromboxane B2 Express EIA kit-monoclonal, Cayman Chemical Company, USA). Comparison between categorical variables was performed by γ²test and the threshold of serum TxB2 has been defined with ROC curve considering Controls and Controls-ASA TxB2 levels and obtaining a cut-off value of TxB2 to define the “aspirin resistance”. Results. All Controls-ASA (100%) had suppressed AA-aggregation (<10%), while 22 MPN-ASA (26.5%) had not (80 ± 16%). No statistical difference was found between Controls and MPN basal or between the MPN-ASA and Controls-ASA. TxB2 production was significantly reduced in Controls-ASA compared to Controls (p <0.0001) as well as in MPN-ASA compared to MPN-basal (p = 0.04); however, MPN-ASA had significantly higher levels of TxB2 than Controls-ASA (p < 0.0001).

0219 RELATIONSHIP BETWEEN METHYLENETETRAHYDROFOLATE REDUCTASE POLYMORPHISMS (C677T AND A1298C) AND METHOTREXATE METABOLISM AND TOXICITY
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Background. Pharmacogenetic is a promising tool for therapy personalization because there are several single nucleotide polymorphisms (SNPs) that can give rise to differing responses to drugs. Methotrexate (MTX) is a structural analogue of folic acid that blocks the enzyme dihydrofolate reductase, inhibiting purine metabolism and causing elevation of homocysteine levels and in several cases, toxicity. The enzyme methylenetetrahydrofolate reductase (MTHFR) catalyses the irreversible conversion of 5,10-methylene tetrahydrofolate to 5-methyltetrahydrofolate. Both C677T and A1298C SNPs are associated with MTHFR activity and consequently, to increased levels of homocysteine. Aims. Analyze the relationship between the presence of A1298C and C677T MTHFR polymorphisms and the metabolism and the toxicity caused by the MTX administration to haematologic patients. Materials and Methods. All included patients were treated according to standardized protocols (HD-MTX). We evaluated 67 MTX infusion courses administered to 17 subjects, aged 17 to 65 years (mean: 45 years). Five out of these patients were affected with ALL, 11 with NHL and 1 with myeloma. MTX concentrations were determined at 12, 36 and 60 hours post-drug infusion, and after, every 24 hours until undetectable MTX levels. We used a Dimension RXL

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Fourier transform infrared spectroscopy is used to monitor molecular changes in leukemia and other cancer types. FT-IR technique provides a method for analyzing drug resistance related structural changes in leukemia and other cancer types.
Table 2.

<table>
<thead>
<tr>
<th>Serious adverse event</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
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</tr>
<tr>
<td>ITP</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>Leukocytopenia</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Rash</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>1/5 (20%)</td>
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<tr>
<td>Pneumonia</td>
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<tr>
<td>Thrombosis</td>
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<tr>
<td>Chest pain</td>
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<tr>
<td>Pyrexia</td>
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</tr>
<tr>
<td>Paresthesia</td>
<td>1/5 (20%)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
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</tr>
<tr>
<td>Retinal occlusion</td>
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<tr>
<td>Gastroenteritis</td>
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</tr>
<tr>
<td>Pancreatitis</td>
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<tr>
<td>Nephritis</td>
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</tr>
<tr>
<td>Pericarditis</td>
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</tr>
<tr>
<td>Pulmonary edema</td>
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<tr>
<td>Thyroid dysfunction</td>
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<tr>
<td>Hypersensitivity</td>
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<tr>
<td>Hypertension</td>
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</table>

0222

PHASE II RESULTS OF THE FIRST-IN-CLASS ANTI-RHD ANTIBODY MIXTURE, ROZROLIMUPAB IN PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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Background. Rozrolimupab is a recombinant antibody mixture of 25 fully human monoclonal antibodies, designed to capture the natural diversity of the human antibody response to RhD as a modern counter-part to the plasma-derived anti-Rhd immunoglobulins currently used in the treatment of ITP. Aims. To investigate the safety and efficacy of a single dose of rozrolimupab in RhD positive, non-splenectomized patients with ITP. Methods. Following informed consent patients were enrolled in this dose escalation (75 µg/kg - 350 µg/kg), multicentre, open label trial. Inclusion criteria included confirmed presence of thrombocytopenia with two individual pre-dosing platelet counts < 30 x 109/L. The patients received a single iv dose of rozrolimupab, were followed for 6 weeks and evaluated for safety and efficacy. Response was defined as platelet count ≥ 30 x 109/L and increase in platelet count from baseline by > 20 x 109/L at 7 days after dosing. Results. Four dose groups comprising 36 patients have been evaluated so far: 75 µg/kg (11 patients), 100 µg/kg (10 patients), 125 µg/kg (10 patients) and 150 µg/kg (5 patients). The four cohorts differed in a number of baseline characteristics including distribution by sex (3, 9, 6 and 4 females), mean platelet count before trial entry (15, 26, 16 and 22 x 109/L) and median time from first ITP diagnosis (68, 20, 5 and 8 months). In the individual dose groups, up to 70% of patients responded including one of the seven patients who had baseline platelet counts below 10 x 109/L. All reported adverse drug reactions (72.8%), except for a severe event of headache in the 100 µg/kg dose group, were of mild or moderate intensity. The reactions included pyrexia (E=2), decreased haemoglobin (E=5) and more frequent (E=6) events of headache. Laboratory data showed that haemoglobin values decreased in all patients indicating biological activity but generally, the values reverted towards baseline during the course of the trial. Three patients had a haemoglobin decrease of ≥ 2 g/dl; the biggest drop (3.1 g/dl) was categorized as a serious adverse event possibly related to rozrolimupab. The event was mild and not associated with serious bleeding and the patient recovered without receiving treatment. Conclusions. Rozrolimupab is well tolerated with no unexpected toxicities and shows preliminary signs of clinical activity. Beneficial effect of this agent in patients with ITP should be further evaluated.

0223

INTERIM RESULTS FROM AN INTERNATIONAL, MULTI-CENTER, SINGLE-ARM STUDY EVALUATING THE SAFETY AND EFFICACY OF ROMIPLOSTIM IN ADULTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP)

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3 Princess Alexandra Hospital, Queensland, Australia
4 University La Sapienza, Rome, Italy
5 Western Pennsylvania Cancer Institute, Pittsburgh, United States of America
6 Complexo Hospitalario Universitario, A Coruña, Spain
7 Fakultni Nemocnice Kralovske Vinohrady, Praha, Czech Republic
8 Insbruck Medical University, Innsbruck, Austria
9 Erasmus Medical Center, Rotterdam, Netherlands
10 Amgen (Europe) GmbH, Zug, Switzerland
11 Aymen Limited, Cambridge, United Kingdom

Background. Romiplostim is recommended for second- and third-line treatment of chronic ITP in adults. This study, conducted in Europe, North America and Australia, investigated romiplostim in adults with ITP of varying duration and severity. Aims. To expand the understanding of the safety and efficacy of romiplostim in adult ITP patients. Methods. Eligibility criteria were broad: patients ≥18 years of age, who had received prior ITP therapies (current amendment: ≥1, previous amendments: ≥2), with low platelet counts (current amendments: ≤ 30 x 109/L, previous amendments: ≤10, 20 x 109/L). The only excluded comorbidities were: hematological malignancy, myeloproliferative neoplasms, MDS and bone marrow stem cell disorder. Romiplostim was initiated at 1 µg/kg/week, with dose adjustments allowed to maintain platelet counts ≥50 x 109/L. The primary endpoint was incidence of adverse events (AEs) and antibody formation. Secondary endpoint was platelet response, defined as either (1) increase of baseline counts by ≥50 x 109/L or (2) ≥20 x 109/L increase from baseline. As of April 2009, 235 patients had enrolled and received at least one dose of romiplostim. Of these, 77% remained on study (121/235) or had completed the study (61/235); 23% (53/235) had...
Table 1. Pre- and on-treatment bone marrow examinations.

<table>
<thead>
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<th>Non-MMF (n)</th>
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Reticulin fibrosis was observed in 85% of patients, which was similar to that reported in a previous study. Nine patients died; 2 deaths (haemolysis, aplastic anaemia) were considered treatment-related. One event of mild (1+) reticulin fibrosis, occurring 3 weeks after last romiplostim dose, was considered serious and treatment-related. No neutralizing antibodies to romiplostim or TPO, or hematopoietic malignancies or MDS events were reported. Approximately 90% of patients achieved each of the platelet response definitions [(1): 86%; (2): 91%; median (Q1, Q3)] treatment duration was 18 (7, 39) weeks (maximum 201 weeks), with a total of 7288 subject-weeks on study. Incidence and type of AEs were consistent with previous studies. Nine patients died; 2 deaths (haemolysis, aplastic anaemia) were considered treatment-related. One event of mild (1+) reticulin fibrosis, occurring 3 weeks after last romiplostim dose, was considered serious and treatment-related. No neutralizing antibodies to romiplostim or TPO, or hematopoietic malignancies or MDS events were reported. Approximately 90% of patients achieved each of the platelet response definitions [(1): 86%; (2): 91%; median (Q1, Q3)]

Methods. This retrospective study included ITP patients receiving treatment with Tpo-RA at the Platelet Disorders Center, The New York Presbyterian Hospital (NYPH), USA, having at least one BM biopsy (BMB) performed prior to or during treatment with Tpo-RA. All BMB and aspirates were performed as part of the standard follow-up practice of patients on Tpo-RA. BM morphology, cytogenetic and flow-cytometric (FCM) examinations were performed in the Pathology Department of NYH. Histological sections were stained with H&E, Gomori stain for reticulin and trichrome stain for collagen; fibrosis was graded according to the European Consensus Classification into 4 grades. Results. 77 BMB were available from 49 ITP patients (median age 50 years; 29 females 59%) treated with various thrombopoietic agents (see table). Ten patients had a pre-treatment BMB, of whom eight had on-treatment biopsies. The grade of reticulin in these eight patients was unchanged from grade MF-0 to MF-1 in 5 patients, decreased from MF-1 to MF-0 in one patient and remained unchanged (MF-1) in two, after initiation of Tpo-RA. Median time from Tpo-RA initiation to first BMB was 1.3 years (IQR 1.0-1.8). Thirty-eight of 45 patients (85%) had greater than MF-0; of these 4 (9%) had MF-2 and 3. Median duration of treatment to second BMB in 16 patients was 3.0 years (IQR 2.3-4.3). From first to second BMB, reticulin grading increased by at least one grade in 6, remained unchanged in 8, and decreased in 2 with ongoing treatment with Tpo-RA. Out of all on-treatment biopsies, 10 patients had BMF with grade 2 or (one) 3 MF-grade. Cytogenetic analysis was performed in 50 of the BM examinations; 2 had abnormal karyotypes (see table). All 26 FCM revealed normal immunophenotypes. Summary/Conclusions. At a median duration of 1.3 years on treatment with Tpo-RA the proportion of patients having reticulin deposition in their marrows was 85%, which appears to be higher than that reported in the literature in ITP patients unexposed to Tpo-RA (40%) (Ettrup et al. Am J Hematol. 2010; 85:930-934) and in pretreatment BMB in this material (50%). Further increment in reticulin was observed in >30% of patients who continued treatment. No serious cytogenetic of immunophenotypic abnormalities emerged during treatment with Tpo-RA. Based on these results, regular follow-up with BM biopsies is recommended during treatment with Tpo-RA.
Figure 1. 6-month event-free survival (LogRank= 2.4; p= 0.1).

**Figure 2027**

Splenectomy in immune thrombocytopenia: Results of 73 cases with a median follow-up of 22 years

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**Background.** Splenectomy is still acknowledged as the gold-standard treatment of refractory immune thrombocytopenia (ITP). Recently, new medical therapies (anti-CD20 and thrombopoietin mimetics) have entered into clinical practice, encouraging a generalized tendency to delay splenectomy. Consequently, the importance of Splenectomy and safety of splenectomy in the long-term is substantial. **Patients and Methods.** We retrospectively analyzed the data of 73 ITP patients who underwent laparotomic splenectomy between 1963 and 1998 and have now a minimum follow-up of 10 years (median, 22 years; range, 10-47). **Results.** Overall, 73 ITP patients followed at our institution have now a minimum observation time of 10 years after splenectomy. Sixty-six percent were women; median time from diagnosis to splenectomy was 13 months (range, 0-254) and median age at splenectomy was 35 years (range, 6-65, with 12 patients younger than 16). Nine patients (12%) underwent splenectomy front-line; the other patients were splenectomized after failure of at least one course of medical therapy (prednisone alone or in combination with azathioprine and/or immunoglobulin). Overall, 66 patients (90%) achieved a complete response (platelet count >100x10⁹/L) after splenectomy and in 44 cases (66%), the response was stable at last contact. Twenty-two patients (33%) lost the response during the follow-up. Nine patients (41%) experienced a very early relapse (within 30 days from splenectomy), while in the remaining 13 patients, relapse occurred after a median time of 40 months (range, 4-256), for a relapse-free survival of 64% at 20 years (Figure 1). Three patients (4%) achieved a response (platelet count >50x10⁹/L), while 4 patients (6%) were refractory. Overall, 28 patients (34%) needed further treatment after surgery. At last contact, 57 patients (78%) were in complete response (15 of whom, thanks to medical treatment post-splenectomy), 3 patients were in response and 13 patients had a platelet count <50x10⁹/L. Eight patients (11%) remained in...
on-demand steroid therapy after a median time of 12 years (range, 3-24) from splenectomy. Forty-two hemorrhagic events (7 of which grade 3-4 WHO) were observed in 17 patients (23%), after a median time of 83 months. Fourteen patients (19%) experienced one or more infectious episodes after surgery, which were severe (pulmonary) in 9 cases and were observed late in the follow-up (median time from splenectomy to the event, 16.7 years, range 2.8-21.7). Nine patients died for causes unrelated to ITP (median age, 74 years; range 43-85).

Conclusions. Splenectomy confirmed to induce a stable remission in 66% of ITP patients in the long-term. Relapse rate was higher in the first months, with sporadic relapses occurring even 20 years after surgery. The incidence of late severe infectious complications was not negligible, probably due to the fact that most patients did not receive prophylactic vaccinations.

0229
AN OPEN-LABEL EXTENSION STUDY EVALUATING THE SAFETY AND EFFICACY OF UP TO 3.5 YEARS OF ROMIPLOSTIM IN THROMBOCYTOPENIC JAPANESE PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA (ITP)

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Background. Chronic ITP is characterized by increased platelet destruction and decreased platelet production. The peptibody romiplostim increases platelet counts by binding to and activating down-stream signaling of the thrombopoietin receptor. Aims. To examine the safety and efficacy of long-term romiplostim use in Japanese patients with chronic ITP. Methods. Patients from a phase 2 open-label study and a phase 3 randomized study could choose to enroll in an open-label extension study. If patients enrolled within 12 weeks of the previous study and had a platelet increase ≥20x10^9/L from baseline once during the 15-week treatment period in the previous study, the romi-
The combination of three dexamethasone cycles and rituximab yields high response rate in previously treated immune thrombocytopenia (ITP)

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Introduction. ITP is characterised by an immune-mediated destruction of platelets and impaired platelet production. The aim of treating ITP is to achieve and maintain elevation of platelet count. Dexamethasone and rituximab are effective in elevating PC and are widely used in the treatment of ITP. Sustained response rates (RR) achieved by multiple cycles of dexamethasone given upfront looked superior to single cycle (85% vs 45%), while RR for rituximab in previously treated patients is about 60% with 40% of complete responses (CR). Recently, combination of multiple cycles of dexamethasone and rituximab in previously treated ITP has not been reported previously. Aims: This pilot study aimed to determine the efficacy and safety of combining three cycles of dexamethasone to rituximab in previously treated patients with ITP. Methods: A retrospective study that enrolled patients with ITP. Patients were followed every 6 months for 1 year. Results: Data at month 6 were available in 132 patients. Median platelet counts at month 6 was 121x109 (range 67-217)/L. At this time, only 13 patients experienced clinical manifestations: skin bleeding (53.8%), oral mucosa bleeding (38.5%) and metrorrhagia (25.1%). Thirty-seven patients underwent a second-line treatment. Mean time between lines was 26.5±0.8 days. The most frequent second-line treatments included rituximab (24.5%), romiplostim (18.9%) and corticosteroids (10.8%). The data was insufficient to assess the response. Splenectomy occurrence was observed in later lines. Conclusion: This interim analysis, which includes a greater recruitment than expected, shows heterogeneity in diagnostic procedures to exclude secondary etiology in patients with thrombocytopenia among the participating hospitals. Moreover, although the first-line treatment was homogeneous and showed a remarkable response rate, the second-line treatments were heterogeneous and further observations that more homogeneous protocols are needed for the management of ITP patients.
Table 1. Patients’ characteristics.

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<tr>
<td>N=21</td>
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<td>Median age in years (Q1, Q3)</td>
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<td>Median platelet count (Q1, Q3)</td>
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20-49 and 133 X 10^9/L (IQR 35-277) respectively. At the last follow-up, 11 patients (52%, 95%CI 30-74) were in CR and 2 (9.5%, 95%CI 1-30) were in PR resulting in total RR of 62% (95%CI 38-82). There was no statistical difference in the CR rates in relation to the disease duration of <1 or ≥ 1 year. During the first 6 months, four grade-3 and one grade-4 AE occurred in two patients. In addition 11 bleeding episodes were recorded in four patients, three of whom were non-responders. Summary/Conclusion. In previously treated patients with persistent, chronic and refractory ITP, the suggested combination yielded a CR rate of 52%, which is comparable to that reported in previously untreated patients by Zaja et al. Four grade 3/4 AE occurred in 2 (10%) patients. 75% of the patients adhered to the designated treatment regimen. The retrospective nature, small sample and short follow-up are the main limitations of this trial. In conclusion, a regimen of rituximab and three 4-days cycles of dexamethasone appears effective, safe and tolerable. This combination merits further exploration in a prospective clinical trial.

References
0234
CYTOKINES AND CHEMOKINES IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (ITP) TREATED WITH TPO-RECEPTORAGONISTS (TPO-RA) COMPARED TO HEALTHY CONTROLS
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Background. Thrombopoietin receptor agonists (TPO-RA) are new treatment modalities for chronic ITP, exerting their effect by stimulating platelet production. Treatment with TPO-RA has been preliminarily shown to increase suppressive activity of regulatory T-cells, but much remains to be learned about the influence of TPO-RA on the immune system. Aims. In this study, the production of a panel of inflammatory cytokines and chemokines in chronic ITP patients treated with TPO-RA was investigated and compared to healthy controls. Methods. Cytokines and chemokines in EDTA-plasma samples from 22 ITP patients treated with TPO-RA (16 females, median age 51 years, IQR 33-58 years, median platelet counts 59x10^9 /L, IQR 30-100x10^9 /L) and 7 healthy controls (2 females, median age 35 years, IQR 21-38 years) were analysed by immuno-bead-based multiplex assay. Results. Elevated levels of soluble CD40 ligand (sCD40L) (median 411 vs. 222 pg/mL, p=0.005), tumor necrosis factor alpha (TNFα) (median 4.0 vs. 0 pg/mL, p=0.000), interleukin-1-receptor antagonist (IL-1ra) (median 915 vs. 325 pg/mL, p=0.001), chemokine (C-X-C motif) ligand 10 (CXCL10) (median 55 vs. 16 pg/mL, p=0.000), CXCL11 (median 52 vs. 23 pg/mL, p=0.000), chemokine (C-C motif) ligand 4 (CCL4) (median 30 vs. 8 pg/mL, p=0.008) and IL-8 (median 5.6 vs. 1.7 pg/mL, p=0.016) were found in ITP patients, with no differences in IL-6, CCL-2 or CCL5. However, a positive correlation between the increase from pre-treatment to on-therapy platelet counts and levels of CCL5 was observed (p=0.01). Correspondingly, a negative correlation between the increase in platelet counts and levels of CXCL11 was observed (p=0.01). No other correlation between increase in platelet counts and cytokine levels were detected in this study. We also found a 3-fold increase in the median ratio of the Th1-associated chemokine CXCL10 compared to the Th2-associated chemokine CCL2 (median ratio 0.56 vs. 0.19, p = 0.001). Summary and Conclusion. The inflammatory mediators sCD40L, CXCL10, CXCL11, CCL4 were significantly increased as compared to healthy controls. This was also observed for TNFα and IL-8, although concentrations were low. CCL5 and IL-6, often associated with acute inflammatory reactions, were not elevated in this series. However, levels of CCL5 correlated to the increased platelet counts following TPO-RA treatment. Increased CXCL10/CCL2 ratio have previously been reported in treatment-naive ITP patients, and is associated with a Th1-predominant active immune response. In our series, ITP patients had higher CXCL10/CCL2 ratios than healthy controls, despite successful treatment with TPO-agonists. Interestingly, levels of CXCL10 inversely correlated to the increase in platelet counts obtained by treatment with TPO-RA. Platelets are associated with inflammatory processes involving both the innate and the adaptive immune compartment. It can be hypothesized that TPO-agonists, i.e. by increasing platelet production, exert a larger than expected effect on the immune compartment in these patients. In this study, we found elevated levels of a range of inflammatory mediators in TPO treated ITP patients compared to healthy controls. Further studies are needed to fully understand the apparent influence of TPO agonists on the immune system in ITP.

0235
ROMIPLOSTIM THERAPY IN CHILDREN WITH REFRACTORY CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA
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Background. Romiplostim, a thrombopoiesis-stimulating peptibody, represents a new therapeutic option in adult refractory chronic immune thrombocytopenic purpura (ITP). There are lacking studies about romiplostim use in pediatric chronic ITP. Aims. This study aimed to assess the short term efficacy and safety of romiplostim in children with refractory chronic ITP. METHODS: Eight patients with chronic ITP refractory to standard lines of therapy were recruited from the Pediatric Hematology Unit, Children's Hospital, Ain Shams University, Cairo, Egypt. One patient was initially excluded because of increased bone marrow reticulin. Therapy was initiated in 7 patients, aged 3.4 years-15.2 years (median 5.5 years), and the disease duration ranged from 15 months-7.8 years (median 2.4 years), none was splenectomized. Romiplostim dose was started as 1µg/kg/week and dose was escalated by 1 µg/kg/week according to platelet count. The duration of therapy varied between 1 week to 22 weeks (median 12 weeks). Results. Results revealed that 4 out of the 7 patients achieved variable response. Four patients demonstrated rapid increase in platelets counts when pulse steroid therapy was added, achieving rapid control of serious bleeding in three patients. Most reported adverse events were mild and transient including acute nasopharyngitis in 2 patients, epistaxis in 1 patient, but one patient developed moderately severe wheezy chest necessitating hospitalization. Conclusions. These results revealed variable response rate in children with chronic ITP to romiplostim therapy; addition of steroids especially in emergency bleeding situations could potentiate romiplostim thrombopoietic effect even in patients initially refractory to steroids. Romiplostim safety and efficacy in pediatric ITP needs further long-term studies.

0236
MORTALITY DURING CLINICAL STUDIES OF ELTROMBOPAG IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA
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Background. Disease- and treatment-related mortality in adult patients with chronic immune thrombocytopenia (ITP) is the subject of ongoing investigation. Reports suggest a rate of 0.3%-12%, increasing to 8%-16% in patients with refractory disease and to 15.7% for refractory patients following splenectomy failure. Cohen reported an incidence of fatal bleeding events of 0.012±0.038/patient year (PY); life expectancy was reduced for patients with persistent thrombocytopenia and for older patients, with 5-year rates of fatal bleeding events of 2% and 47.8% for patients <40 years and >60 years, respectively. Similar findings were reported by Portielje, who noted that the most common causes of death were bleeding and infection in almost equal proportions. Eltrombopag is an oral thrombopoietin receptor agonist approved for chronic ITP. Aims. To describe mortality in adult patents with chronic ITP in eltrombopag clinical studies. Methods. Mortality among 494 adult patients with chronic ITP exposed to placebo or eltrombopag were analyzed from 5 clinical trials: two 6-week, placebo-controlled studies (TRA100773A/B: eltrombopag, n=164; placebo, n=67); RAISE, a 6-month, placebo-controlled, phase 3 study (eltrombopag, n=135; placebo, n=62); REPEAT, an open-label study of 66 patients treated intermittently with eltrombopag in three 6-week cycles; and EXTEND, an ongoing extension study with 299 patients from prior eltrombopag trials receiving eltrombopag for up to 2 years. Results. Eight patients (1.6%) have died (eltrombopag, n=2; placebo, n=1), with an overall exposure period of 584.4 PYs for eltrombopag and 35 PYs for placebo. The overall mortality rate for patients who died on therapy or within the subsequent 8 weeks was 0.0077/PY (95% CI 0.0025, 0.018). Two patients died 107 and 199 days following the last dose of therapy: one patient was treated with eltrombopag for 13 months with a history of ITP and Waldenstrom's macroglobulinemia, had a final platelet count <20 000/µL, and died of multiorgan failure secondary to hemorrhage following severe bruising. The second patient was treated with eltrombopag for 1 week, and died at home following a fall and sustained head trauma. Conclusions. Mortality was observed in two of 494 patients (0.4%) treated with eltrombopag in clinical studies; the observed rate is not significantly different from placebo. No deaths were noted in placebo or eltrombopag groups in clinical studies lasting >8 weeks.
be related to study medication. Fatal hemorrhage occurred in 3/494 patients: two, treated with eltrombopag, had never achieved a response and died 55 and 107 days following their last dose of eltrombopag; a third patient, treated with placebo, died of an intracranial hemorrhage. Other deaths include one each of cardiorespiratory failure, multi-organ failure, motor vehicle accident, pancreatic cancer, and unknown (died at home with no autopsy).

**Conclusions.** No discernible pattern in cause of death is apparent, other than fatalities due to bleeding events in patients with persistently low platelet counts. The mortality rate across the eltrombopag trials is lower than that reported in the literature. These data need to be interpreted cautiously as patients enrolled in clinical trials are subjected to specific monitoring schemes not necessarily implemented in clinical practice.

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### References


**0237**

**SUSTAINED HEMOSTATIC PLATELET COUNTS IN ADULT PATIENTS WITH PRIMARY IMMUNE THROMBOCYTOPENIA (ITP) FOLLOWING CESATION OF ROMIPLOSTIM - FOUR EUROPEAN CASE STUDIES**

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**Background.** Romiplostim is recommended for second- and third-line treatment of chronic ITP in adults. While often perceived as a chronic, long-term treatment, previous data suggest some patients maintain hemostatic platelet counts when treatment stops. **Aims.** To describe individual cases of patients maintaining hemostatic platelet counts following cessation of romiplostim. **Methods.** We describe four patients with long-term (6-49 years), chronic ITP, and refractory to previous treatment options, including splenectomy, corticosteroids, IVlg, anti-D and rituximab. All received romiplostim in clinical trials, initiated at 1-3 µg/kg/week and with dose adjustments allowed to maintain platelet counts above 50 x 10^9/L. Romiplostim was withheld when counts rose above a pre-specified threshold (400 or 450 x 10^9/L) and reinitated at a lower dose when counts fell below 200 x 10^9/L. Following completion of the relevant trial, patients were managed as per routine clinical practice. **Results.** Patient 1 completed a 24-week trial, during which platelet counts increased to normal levels. Following cessation of romiplostim, counts fell slightly and a single dose of IVlg was administered. Thereafter, hemostatic platelet counts were maintained without any ITP therapy for over 4 years. Patient 2 experienced high platelet counts after 1 year of romiplostim treatment and romiplostim was withheld. At the last recorded contact, patients 1, 2 and 4 had maintained platelet counts >50 x 10^9/L for 9 months-4.5 years in the absence of any ITP therapy or bleeding episodes. **Summary/Conclusions.** Dose adjustment rules allow romiplostim to be discontinued when hemostatic platelet counts are reached. This report of sustained hemostatic counts following romiplostim cessation provides evidence that romiplostim can be a short-term treatment in some adult ITP patients, including those with long-term, chronic disease. While the exact incidence of such cases is unknown, additional anecdotal evidence may provide more insight.

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**0238**

**INTRAMUSCULAR USE OF ANTI-D GAMMAGLOBULIN IN PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA: A SINGLE CENTER EXPERIENCE**

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**Background.** Treatment guidelines support the intravenous administration of anti-D gammaglobulin as an option in Rh positive patients with a diagnosis of immune thrombocytopenic purpura (ITP). However, in Spain this drug is only available for intramuscular (IM) injection. This is the reason why we used the anti-D immunoglobulin intramuscularly in our patients. **Aims.** In this study we show our experience with IM anti-D gammaglobulin, when indicated, in the treatment of ITP patients. Our purpose was to evaluate the efficacy and safety of this therapeutic option. **Methods.** We have analyzed 41 patients with ITP treated with IM anti-D gammaglobulin in our institution over the past 20 years. The unitary dose was 900 micrograms repeated on days 1, 2 and 4 as the initial dose (loading dose). After one week, another loading dose was administered if necessary, and eventually further single doses as maintenance. The current consensus guidelines published in 2009 by the IWG were used to assess the response to treatment, especially the time to achieve a response and a complete response, and the number of doses required to obtain them. **Results.** Thirty of 41 patients were evaluable (median age 47 years, range 16-85; 19 women and 11 men). The exclusion criteria were a diagnosis other than ITP or the discontinuation of treatment before completing at least one loading dose. In most cases, anti-D gammaglobulin was administered as second or third line treatment, with median prior lines 2 (range 0-4). Corticosteroids were the initial therapy in 28 patients, most often associated with intravenous immunoglobulins (17 cases). The indications for starting anti-D were: relapse after other lines of treatment in 14 patients (47%), refractoriness to initial therapy in six cases (20%), and as maintenance approach in eight (27%). One patient received anti-D as first line therapy and another one because of toxicity to previous treatments. The mean platelet count before starting anti-D was 67 x 10^9/L. The number of patients who achieved complete response criteria (platelet count > 100 x 10^9/L) was 23 (77%), while two patients failed to reach it. Complete response criteria were not applicable in five cases because the initial platelet count was above 100 x 10^9/L. No adverse effects related to drug (especially any bleeding complication associated with the intramuscular route of administration) were registered. **Conclusions.** Although current guidelines do not include the IM administration of anti-D gammaglobulin, our experience demonstrates that this is an effective, safe, and economical option, with the added advantage of an outpatient administration.
Background. In 2010 an International Multicentric Cooperation proposed a new, comprehensive cytogenetic scoring system for primary MDS (Hasse et al., EHA 2010). Aims. The present study was aimed at assessing whether this new cytogenetic scoring system could improve the prognostic stratification of 631 consecutive de novo MDS patients, who were observed in the period January 1990-January 2010. Methods. Inclusion criteria were: primary MDS, age ≥16 years, bone marrow blast cell percentage <20%, supportive therapy only. Univariate and multivariate analyses concerning overall survival (OS) and MDS/AML progression were performed. Median follow-up was 19.6 months (mo) (inter-quartile range, IQR, 7.3-46.3). At the time of the analysis 160 pts (25.3%) had died after a median follow-up of 18.7 mo. (IQR 8.4-48.1) and 137 pts (21.7%) had experienced MDS/AML progression after a median time of 14.6 mo. (IQR 8.9-34.2). Results. There were 49 sulfonates and 54 females whose median age was 65.3 yrs (IQR 56.6-72.5). According to WHO 70 pts (11.1%) were RARS, 122 (19.3%) RA, 25 (3.9%) CRML with ringed sideroblasts (RCMDs), 149 (23.6%) RCMD, 38 (6.0%) 5q- syndrome, 9 (1.4%) unclassifiable MDS (u-MDS), 102 (16.1%) RAEB-1 and 116 (18.4%) RAEB-2. According to IPSS 177 pts (28.1%) were low-risk, 255 (40.4%) int-1 risk, 139 (22%) int-2 risk and 60 (9.5%) high-risk. Three-hundred fifty-three (55.9%) pts presented an abnormal karyotype: 260 (41.2%) carried a single chromosomal defect, 46 (7.3%) carried two defects and 47 (7.4%) >three defects. Based on the new cytogenetic scoring system, 14 pts (3.8%) were considered very good risk, 392 (62.1%) good risk, 162 (25.6%) intermediate risk, 29 (4.6%) high-risk and 34 (5.3%) very high-risk. Two-years OS was 91.0% (95% CI: 50.8-98.7) for the very good-risk category, 87.0% (95% CI: 82.7-90.6) for the good-risk category, 72.0% (95% CI: 63.2-80.0) for the intermediate-risk category, 54.8% (95% CI: 29.7-74.1) for the high-risk category and 9.4% (95% CI: 0.6-32.9) for the very high-risk category (p<0.0001). Multivariate analysis, which compared each category to the very good, resulted in a Hazard Ratio of 1.7 for the good-risk category; 2.8 for the intermediate-risk category; 4.0 for the poor-risk category and 16.3 for the very-poor risk category (p<0.0001). Two-years PH was 90.9% (95% CI: 50.8-98.6) for the very good-risk category, 79.0% (95% CI: 74.0-83.3) for the good-risk category, 54.7% (95% CI: 45.3-63.2) for the intermediate-risk category, 46.1% (95% CI: 22.7-66.7) for the high-risk category; no patient of the very high-risk category was surviving at this time (p<0.0001). Multivariate analysis resulted in a Hazard Ratio of 2.0 for the good-risk category; 3.9 for the intermediate-risk category; 4.4 for the poor-risk category and 15.5 for the very-poor risk category (p<0.0001). Six multivariate models were compared by means of Akaike Information Criterion (AIC). To predict OS, the best models included age, peripheral cytopenias, WHO classification, and either new cytogenetic categories or number of chromosomal abnormalities (AIC=1651 and 1622); to predict PH, the best models included the same variables (AIC=2454 and 2448). Conclusion. The new cytogenetic scoring system is effective in improving the prognostic stratification of pts with de novo MDS.

Monosomy 7 Syndrome with Rapid Onset and Poor Survival

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Background. Myelodysplastic syndromes (MDS) are a group of clonal hematologic neoplasms with different risk for progression to acute leukemia and survival. The current 2008 WHO classification for MDS
was built in an attempt to improve the prognostic value of the previous FAB classification. The 2008 WHO classification is based on the percentage of blast cells in bone marrow (BM) and peripheral blood (PB), the type and degree of dysplasia, the proportion of ring sideroblasts, and number of cytopenias. However, the reproducibility of WHO classification is still unclear. The aim of this study was to analyze the interobserver concordance between four experienced morphologists from three different centers and to define potential diagnostic difficulties. Methods. Smears from PB and BM samples (stained by May Grunwald-Giemsa and iron) from 50 patients with well established diagnosis of MDS were blindly analyzed by 4 observers. The assessment of myelodysplasia and blast cell enumeration was performed as recommended by the WHO criteria (Swerdlow et al, 2008). The degree of correlation between observers in the percentages of blast cells in BM and PB and ring sideroblasts was analyzed by Pearson correlation and inter-observer agreement index in the diagnostic morphological subtype (7 categories) and degree of dysplasia (3 categories; <10%, 10 - 59%, > 40%) was studied by using the generalized kappa statistic for multiple raters. Results. The degree of correlation between observers for the proportion of blast cells in BM (R, .44 - .89; P, .002 - <.0001) and PB (R, .31 - .72; P, .03 - <.0001) and ring sideroblasts in BM (R, .77 - .94; P, <.0001) was statistically significant in all the subtypes. The kappa coefficient for dysgranulopoiesis, erythroid, and megakaryocyte dysplasia was .40, .15, and .41 respectively. The kappa value for dysplastic subtype was .39, ranging from .17 for refractory cytopenia with unilineage dysplasia to .66 for mixed MDS/myeloproliferative disorders (chronic myelomonocytic leukemia subtype). Conclusions. These results suggest that there is a good correlation between observers in quantification of the percentage of blast cells in BM and PB and ring sideroblasts and that the degree of concordance in the assessment of dysplastic changes is adequate for the granulocytic and megakaryocytic lineages but inadequate for erythroid lineage. However, the apparently accurate concordance for most parameters evaluated did not translate in a good agreement in the final diagnosis classification for severe or advance of agreement which could be due to an arbitrary cut off points in the percentage of blast cells and dysplastic features that determine the different subtypes.

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0243 ACTIVATION OF COMPENSATORY PATHWAYS ARE RESPONSIBLE OF AML SECONDARY TO HIGH-RISK MDS TREATED WITH 5- AZACYTIDINE

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Background. Approximately one-third of Myelodysplastic syndromes (MDS) patients progresses to acute myeloid leukemia. Several studies have shown increased rates of programmed cell death (apoptosis) in marrow cells of patients with low grade/early stage MDS, and a changing interplay of pro-apoptotic and anti-apoptotic signals is central to disease progression. 5-azacytidine (5-AZA) is a DNA methyltransferase inhibitor used in treatment of high-risk MDS. A large clinical trial showed its beneficial in achieving better clinical outcomes and quality of life when compared to supportive care. Despite 5-AZA can delay the progression of MDS to acute myeloid leukemia (AML), by preventing the transformation of tumor suppressor genes. Changes in progression to AML is still too high. Methods. We analyzed 17 patients affected of high risk MDS, treated for 4 months with 5-azacytidine (median age 71 years, M/F=12/5). In 5 cases the sample after 4 cycles of therapy was matched to the sample collected before the therapy start. Bone marrow mononuclear cells were obtained using density gradient centrifugation (Fycoll) under sterile conditions, at the beginning of treatment and after 4 cycles of therapy. All samples were lysed at the same time into 40 ul of lysis buffer containing a 1:1 mixture of 2x Tris-Glycine SDS sample buffer (In vitrogen Life Technologies) and Tissue Protein Extraction Reagent (Fermentis) plus 1.0% beta-mercaptoethanol for 5 min at 100°C. Reverse-phase protein microarray (RPMA) is a reproducible, high-throughput system for protein signal pathway profiling. RPMA were used to quantitatively map 45 cell signaling pathway endpoints, including survival, proliferation, drug resistance, apoptosis, and autophagy. Results. All patients were evaluable for response one month after the 4th cycle. 2 patients were refractory, progressing to AML under treatment, 1 progressed with a concomitant monoclonal gammopathy, and 1 was a late responder (documented response after 7cycles). 5/17 protein endpoints were linked together in the induction of a compensatory pathway inhibited after the treatment with 5-azacytidine, independently from the quality of achieved response. PLCy-1/Tyr783 (p=0.0017), and its up-stream, SrcTyr416 (p=0.002) and downstream target STAT5/Tyr694 (p=0.0017) were increased, without affecting proliferative pathways, such as AKT activation status on Ser473 and Thr403 or mTORSer2448. Comparing pool of samples at diagnosis with the pool of samples after 4 cycles we found an increase of the three main proteins considered markers of autophagy: ATG5 (p=0.0001), Beclin1 (p=0.0056) and LC3B (p=0.0124), independently from the achieved quality of response. The activation occurred downstream mTOR pathway. Since mTORSer2448, AktSer473, AktThr403, ERKThr202Tyr204 were not affected. Conclusion. We identified at protein level two compensatory pathways induced by
5-azacytidine. Since STAT3 is involved also in the transcriptional regulation of HS/H4, herein we provide the molecular rationale for the development of a combination based upon 5-azacytidine+ inhibitors of HDAC. Similarly, autophagy activation can be considered an escape pathway of survival in neoplastic cells. The observation that 5-azacytidine does not affect proliferative pathways, identified as new potential targets (Pl. S Gene) suggests the need to combine 5-azacytidine to anti-proliferative agents, such as Rapamycin or RAD001, in order to target proliferation of neoplastic cells.

0244 METHYLATION OF WNT ANTAGONISTS AND EFFECTS OF AZA TREATMENT ON WNT PATHWAY IN MDS CELLS

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Background. The implication of the Wnt pathway in self-renewal and proliferation of hematopoietic stem and progenitor cells suggests its involvement in the pathogenesis of haematopoietic neoplasias. This signaling cascade is controlled by different Wnt antagonists that interfere with ligand-receptor activating interactions. Among them, members of the DKK and sFRP family, whose activation induces the accumulation of non-phosphorylated β-catenin (NPBC) in the cell nucleus. Interestingly, aberrant methylation of these Wnt antagonists has been demonstrated in different hematological malignancies (acute myeloid leukemia and chronic myeloid leukemia). Objectives. 1. To study the methylation status of the Wnt antagonists: sFRP1, sFRP2, sFRP4, sFRP5 and DKK1 in primary MDS cells. 2. To evaluate the correlation between methylation of Wnt antagonist and activation of the Wnt pathway, analyzing the expression of NPBC using bone marrow MDS cells before and after treatment with azacytidine (AZA).

Methods. We studied bone marrow cells from 24 patients diagnosed with MDS according to the WHO classification: 10 RCMD, 5 RAEB-1, 3 RA and 6 MDS. Median age was 73 yr and M/F was 14/24. After bisulphite treatment, methylation was evaluated by methylation specific PCR (MSP) using primers specific for the methylated and unmethylated alleles of the genes. Bone marrow cells of MDS patients were grown in RPMI 1640 medium supplemented with BSA and treated with AZA 1μM during 48 hours. Expression of β-catenin was studied by confocal microscopy using antibodies against total β-catenin and NPBC. Results. Hypermethylation of the gene promoters was observed for all Wnt antagonist genes. Among the 24 cases, methylation frequencies were as follows: 75% sFRP2, 75% DKK1, 61% sFRP1, 29% sFRP5 and 16% sFRP4. Most MDS patients (92%) showed methylation of at least one gene, ranging from one to 5 methylated genes. Bone marrow cells of the MDS cases with sFRP2 and sFRP4 together with sFRP4 methylated, respectively, were cultured in vitro. After AZA treatment, a reduction of DNA methylation level of sFRP2 and sFRP4 was observed, indicating the decrease in the pathway activity. Confocal microscopy showed a reduction of NPBC in the cell nucleus, clearly indicating that inactivation of the Wnt pathway was provoked by the treatment with AZA. Conclusion. Hypermethylation of Wnt antagonists is a frequent event in MDS and seems to be associated with activation of the Wnt pathway, as demonstrated by the relocation of NPBC in the compartments after AZA treatment.

0245 ALTERED CELL CYCLE PROFILES OF SPECIFIC COMPARTMENTS OF BONE MARROW (BM) CELLS FROM MYELODYSPLASTIC SYNDROME PATIENTS IS ASSOCIATED WITH PROGNOSTIC FEATURES OF THE DISEASE

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Background. Epigenetic aberrations are now well recognized as very frequent and also as early events in the process of malignant transformation. It is very often reported that gene-specific hypermethylation occurs in the context of global hypomethylation. The clinical responses of MDS to drugs that reverse aberrant hypermethylation, such as 5-aza-2-deoxycytidine and 5-azacytidine, suggest that aberrant hypermethylation plays a causative role. Aims. We investigated if DNA methylation by immunohistochemistry in bone marrow trephine biopsy specimens in a cohort of 132 MDS patients comprising all subgroups. Results were compared to an age-matched control group of 47 healthy subjects and to a group of de novo (36) and secondary (20) AML patients. We applied a double staining procedure to discriminate different lineage of positive cells. Methods. Immunohistochemistry was performed on paraffin-embedded sections using anti-5-methylcytosine/5mc antibody. Scoring of immunohistochemistry was evaluated with a four-points scale for both the number of positive tumor cells and their intensity of immunoreactivity. Double immunostainings were performed on histological section for nuclei 5-aza-2-deoxycytidine/5mc and one of four cytoplasmic/cell membrane markers (CD34 for precursor, MPO for myeloid cells, Glycophorin-C for erythroid cells, Factor VIII for megakaryocytes) by using EnVision® GI2 Doublestain System.
Rabbit/Mouse (Dakocytomation): cells showing double stainings between nuclei and cytoplasmic areas or cell membranes. Results. Normal bone marrows showed a low number of cells reactive for 5mc: with double stainings they were recognised as intermediate myeloid MPO-reactive and early erythroid glycophorin-C-reactive precursors accounting for less than 10% of the entire series. Segmented and polymorphonucleated granulocytes and orthochromatic erythroblasts were not stained with 5mc+/CD34+ precursors with double stainings were hardly visualised in sections. Compared to normal bone marrows, MDS and AML cases showed respectively a moderate and a marked increase of positivity for 5mc. Primary AML were characterized by the highest percentage of 5mc+/CD34+ and 5mc+/MPO+ cells, including also some immature cells like segmented granulocytes and bands. 5mc+/glycophorin-C+ cells were few in this group of cases. Secondary AML showed a percentage of 5mc+/MPO+ and 5mc+/glycophorin-C+ and cells and 5mc+/CD34+ cells higher than MDS cases and lower than primary AML. Unilineage and multilineage MDS without excess of blasts showed a mild increase of 5mc+/CD34+ precursors compared to MDS without blasts and normal bone marrows. Differences were statistically significant between AML and MDS cases and between AML and normal marrows. In MDS the 5mc+/CD34+ and 5mc+/MPO+ percentage correlated significantly with the risk score according to the International Prognostic Scoring System. Factor VIII+ megakaryocytes were frequently reactive for 5mc+/CD34+ and 5mc+/MPO+ percentage correlated significantly with bone marrows. Differences were statistically significant between AML and MDS without blasts and normal bone marrows. Unilineage and multilineage MDS without excess of blasts and secondary highest percentage of 5mc+/CD34+ and 5mc+/MPO+ cells, including MDS and AML cases showed respectively a moderate and a marked hardy visualised in sections. Compared to normal bone marrows, morphonucleated granulocytes and orthochromatic erythroblasts were with double stainings they were recognised as intermediate myeloid precursors to characterize the t(3;11)(q13;q14) rearrangement in MDS at the genetic, molecular and functional level. FISH analyses were performed using BAC and fosmid microarrays based karyotyping but there was no evidence for the chromosomal aberrations (copy number variation) and pathological processes originating from hematopoietic stem cells. How-ever, limited information is available regarding the importance of miRNAs for the development and progression of myelodysplastic syndrome (MDS), a heterogeneous group of clonal preleukemic conditions with high risk of transformation into secondary acute myeloid leukemia (AML). Recently, 46 potential miRNA genes located in the human imprinted 14q32 region were suggested to play a role in various patho- The present study was performed to determine whether the determination of global methylation levels may serve as a new predictive marker for therapy response.

**0247**

**OVEREXPRESSION OF THE ILDR1 GENE IN MYELODYSPLASTIC SYNDROMES**

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**Background.** Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic stem cell disorders characterized by ineffective hematopoiesis and a propensity to progress into acute myeloid leukemia. In de novo MDS, chromosomal lesions consist mainly of unbalanced rearrangements and numerical defects whereas balanced structural rearrangements are rare, being observed in fewer than 5% of patients and their prognostic impact remains unknown. **Aims.** We aimed to characterize the t(3;11)(q13;q14) rearrangement in MDS at the genomic, molecular and functional level. **Methods.** One-hundred and fifteen MDS patients at diagnosis were analyzed by conventional cytogenetic analyses performed using BAC arrays and probes selected according to the University of California Santa Cruz database (http://genome.ucsc.edu/). Quantitative real-time PCR (qRT-PCR) experiments were performed using the ABI Prism 7800 Sequence Detection System. Statistical analysis of the relative expression results was performed with the Relative expression software tool (REST). **Results.** Two (1.7%) cases showed a t(3;11)(q13;q14) translocation. FISH experiments detected the presence of the same breakpoints in both patients. UCSC database query showed that no known gene was located on the chromosome 11 breakpoint, whereas 3 genes (CD86, ILDR1, and CASR) with known function were mapped next to the chromosome 3 breakpoint. qRT-PCR experiments showed ILDR1 up-regulation in the patients by a mean factor of 13.775 (p=0.02). Bioinformatic analysis of the chromosome 11 breakpoint region showed the presence of a promoter (892)+2 and a CpG island (CpG 172) at a distance of about 220 Kb from the breakpoint region. **Conclusions.** We reported a novel t(3;11)(q13;q14) rearrangement associated with overexpression of the immunoglobulin-like domain-containing receptor (ILDR1) gene in MDS patients. We hypothesize that the gene upregulation could be mediated by the juxtaposition of regulatory elements next to the ILDR1 gene as a consequence of the chromosomal translocation. ILDR1 expression has been related to the development and/or progression of other solid tumors and it has been detected in infiltrated tissues transformed into advanced grade follicular lymphoma to a high-grade diffuse large B cell lymphoma. The question whether there is a functional link between the clinical features and the ILDR1 gene dysregulation and whether it may have a potential prognostic significance in MDS remains to be established.

**0248**

**UP-REGULATION OF SUBSET OF MiRNAS LOCATED IN 14q32 DOMAIN IN CD34+ BONE MARROW CELLS FROM PATIENTS WITH MYELODYSPLASTIC SYNDROME**

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**Background.** MicroRNAs (miRNAs) are small non-coding RNAs functioning as key regulators of many cellular processes including hematopoiesis. Differential miRNA expression patterns and a number of causative aberrations in miRNA genes have been detected in various pathological processes originating from hematopoietic stem cells. However, limited information is available regarding the importance of miRNAs for the development and progression of myelodysplastic syndrome (MDS), a heterogeneous group of clonal preleukemic conditions. miRNAs are involved in many biological processes including cell differentiation, proliferation, survival, apoptosis, addiction and transformation. These results may provide a molecular explanation for the success in treating MDS patients with hypomethylating agents. Furthermore, it remains unknown whether the determination of global methylation levels may serve as a new predictive marker for therapy response.

**Methods.** Aims. We aimed to characterize the t(3;11)(q13;q14) rearrangement in MDS at the genetic, molecular and functional level. **Methods.** One-hundred and fifteen MDS patients at diagnosis were analyzed by conventional cytogenetic analyses performed using BAC arrays and probes selected according to the University of California Santa Cruz database (http://genome.ucsc.edu/). Quantitative real-time PCR (qRT-PCR) experiments were performed using the ABI Prism 7800 Sequence Detection System. Statistical analysis of the relative expression results was performed with the Relative expression software tool (REST). **Results.** Two (1.7%) cases showed a t(3;11)(q13;q14) translocation. FISH experiments detected the presence of the same breakpoints in both patients. UCSC database query showed that no known gene was located on the chromosome 11 breakpoint, whereas 3 genes (CD86, ILDR1, and CASR) with known function were mapped next to the chromosome 3 breakpoint. qRT-PCR experiments showed ILDR1 up-regulation in the patients by a mean factor of 13.775 (p=0.02). Bioinformatic analysis of the chromosome 11 breakpoint region showed the presence of a promoter (892)+2 and a CpG island (CpG 172) at a distance of about 220 Kb from the breakpoint region. **Conclusions.** We reported a novel t(3;11)(q13;q14) rearrangement associated with overexpression of the immunoglobulin-like domain-containing receptor (ILDR1) gene in MDS patients. We hypothesize that the gene upregulation could be mediated by the juxtaposition of regulatory elements next to the ILDR1 gene as a consequence of the chromosomal translocation. ILDR1 expression has been related to the development and/or progression of other solid tumors and it has been detected in infiltrated tissues transformed into advanced grade follicular lymphoma to a high-grade diffuse large B cell lymphoma. The question whether there is a functional link between the clinical features and the ILDR1 gene dysregulation and whether it may have a potential prognostic significance in MDS remains to be established.

**0249**

**ABERRANT BNP3 EXPRESSION IN MDS CELLS: A POSSIBLE DECITABINE TARGET**

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**Introduction.** Myelodysplastic syndrome (MDS) encompasses a group of clonal hematopoietic stem cell disorders characterized by ineffective
AML patients and normal donors, and the expression levels of BNIP3 during erythroid differentiation of CD34+ cells from normal MDS cells submitted to treatment with 5-aza-2'-deoxycytidine quantitative PCR in total cells. Mononuclear cells from four MDS patients and 4 MDS patients.

Nevertheless, there are as yet no studies of the expression or function of BNIP3 in MDS. BNIP3 expression in MDS is reduced in hematopoietic cell lines and primary leukemia cells and this result was associated to aberrant methylation and histone deacetylation of the transcription start site. BNIP3 expression is also reduced in myeloproliferative neoplasia, indicating that BNIP3 may play a role in the disturbed apoptosis observed in these diseases. Furthermore, BNIP3 was related to the regulation of erythrocyte production through modulation of apoptosis. Nevertheless, there are as yet no studies of the expression or function of BNIP3 in MDS. Aims. The aim of the present study was to characterize BNIP3 expression levels in bone marrow cells from MDS and AML patients and normal donors, and the expression levels of BNIP3 in MDS cells submitted to treatment with 5-aza-2'-deoxycytidine (DAC). Moreover, we evaluated the expression levels of BNIP3 transcripts during erythroid differentiation of CD34+ cells from normal donors and MDS patients. Methods. Bone marrow aspirates were obtained from thirty-four patients with MDS and twenty-eight patients with AML. Samples were collected from patients at the time of diagnosis. Twenty samples from normal donors were used as controls. MDS patients were grouped in low-risk and high-risk according to FAB, WHO and IPSS (Table 1). Gene expression was evaluated by quantitative PCR in total cells. Mononuclear cells from four MDS patients were isolated with Ficoll Hypaque density gradients and treated with 5μM DAC for seventy-two hours. Erythroid-differentiation was performed in CD34+ bone marrow cells from 4 normal donors and 4 MDS patients. Results. We observed a significant decrease in BNIP3 expression of AML and MDS cells compared with normal hematopoietic cells (0.52 [5.27-0.00]; 0.52 [5.25-0.02] versus 1.09 [6.04-0.18], respectively; p<0.05). In MDS, BNIP3 expression was lower in both low-risk and high-risk patients according to FAB and WHO classification, IPSS and number of cytopenias, when compared to normal subjects. Interestingly, among the mononuclear cells submitted to DAC treatment, BNIP3 expression was increased by three fold after DAC exposure in the cells from the two patients that showed lower BNIP3 expression. BNIP3 expression was not modulated during erythroid differentiation in normal and MDS cells. Conclusion. The downregulation of BNIP3 in MDS cells may play a role in the dysregulation of apoptosis in hematopoietic cells leading to ineffective hematopoiesis. The increase in BNIP3 expression after DAC treatment indicates that this gene may be epigenetically inactivated by methylation in this disease and might be a target for DAC treatment. Although it is difficult to explain the pathophysiology of the MDS disease, BNIP3 gene modulation, the study of different pathways is important in order to identity new prognostic markers or therapeutic targets in this disease.

Table 1.

**0250**

**SELECTION BY SEVERE HYPOXIA OF REPOPULATING PROGENITOR CELLS IN PRIMARY MDS BONE MARROW CELL CULTURE**

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Myelodysplastic Syndromes (MDS) are clonal disorders. However, whether the transforming event occurs in a myeloid committed cell or in an earlier progenitor (stem cell) is still not ascertained. Evidence have been accumulated in which senses, but MDS initiating cells must be capable of sufficient repopulating capacity to perpetuate the disease. Objectives. To evaluate the repopulating ability of BNIP3 selected cells in primary MDS bone marrow cultures and characterize the “stemness” of MDS maintaining cells. We evaluated 12 patients with different WHO subtypes of MDS (RARS:5, RAEB-1:3, RAEB-II:3, 1 RA). Mononuclear bone marrow cells were isolated after gradient centrifugation and grown in RPMI 1640 medium supplemented with 20% FBS and a cocktail of cytokines (TPO, FLT3-L, SCF, -IL3). Cells were incubated and selected in Ruskin Concept 400 aneboic incubator, in severe hypoxia conditions by flushing with a perfomred gas mixture (0.5% O2, 5%CO2, 95% N2). Cells were cultured under hypoxic conditions for 10-15 days (LC1), daily counted (Trypan blue) and then recovered from the culture. The stem and progenitor cell potential of these cultures at different times of incubation was explored by transferring cells to growth-permissive secondary cultures in normoxia (LC2), with SCF, -G-CSF, -IL-3, -IL-6, according to the Culture-Repopulating Ability assay methodology (Leukemia, 14:725-9, 2000). The patient was enriched by semisolid medium was also evaluated in parallel in the presence of the same cytokine cocktail. The hypoxic culture system allowed selection of a minute cell population: in 12/12 cases viable cell number was decreased of one log after 10-13 days. In one case we observed, after hypoxia selection, a reduction of CD34 positive cells (0,15% against 9,44%). This population was enriched with CD133 positive cells (91.8% against 85% before selection), and CD38 positive cells were also increased (77% against 45%). Only 2/10 cases showed a significant repopulating ability at day 17 of LC2. In the other 10/10 cases, repopulating ability was apparently absent. Surprisingly, MDS cases presenting blasts in the bone marrow did not show more repopulating cells after selective hypoxic conditions. Although our results are preliminary, we demonstrate that it is possible to select by severe hypoxic conditions primary MDS progenitor cells endowed with repopulating ability. Further characterization (phenotypic and molecular) of these selected progenitors will allow a deeper insight into the biology of this heterogenous group of diseases.

**0251**

**UNSUSPECTED CHROMOSOMAL LESIONS ARE REVEALED BY FISH IN KARYOTYPICALLY NORMAL MDS PATIENTS**

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Background. In MDS the cytogenetic pattern is the most important parameter to predict overall survival (OS) and the risk of MDS/AML evolution, but 40-50% of patients are not informative being chromosomally normal. Aims. Our study evaluated whether probes derived from aCGH studies were truly able to unmask cryptic lesions in chromosomally normal MDS patients and influenced OS and disease evolution. Methods. The 75 chromosomally normal MDS patients analysed came to our observation in the period January 2005-December 2010. They were twenty-five females and forty-eight males, their median age was 64 years (range 22-77). According to WHO classification, 5 patients were classified as RARS, 22 as RAEB-1, 22 as RAEB-II, 22 as RARS, 22 as RCM, 22 as RAEB-1 and 14 as RAEB-2. Considering IPSS score, 24 patients were considered low-risk, 28 intermediate-1 risk and 15 intermediate-2 risk and 6 as high-risk. Median follow-up was 21 months (range 1-66). At the time of the study no patient has died. Overall, 9 transformed into a more advanced MDS subtypes and 11 into AML. FISH probes were chosen based on the frequency of their involvement and their Mb position (UCSC genome browser on Human Mar. 2003 assembly). They were obtained from BACPAC Resources Center at C.H.O.R.I. (Oakland, USA), labelled and...
applied as previously described. We used the following probes: RP11-912D21, RP11-199p12, RP11-269c4 (4q12); RP11-35101 (10q21.3); RP11-144g6 (10q11.2); RP11-122a11 (7q34); RP11-951k18 (8q13.1); RP11-100m20 (4p14); RP11-544h14 (2q33). For i-FISH, cut-off values, obtained from the analysis of 300 nuclei from ten normal samples, were fixed at 10%. Results An abnormal FISH pattern was revealed in 26 patients (56.6%). A single defect was revealed in 19 patients, while 7 patients had more than two deletions (14.2%). Single deletions (61.5%) presented a 19q13.2 deletion, 7 (26.9%) a deletion of band 14q13.2, 4 (15.4%) a deletion of band 17q12.2, 4 (15.4%) a deletion of band 1p13, 3 (11.5%) a deletion of band 10q11.22, 3 (11.5%) a deletion of band 7q34, 2 (7.7%) a deletion of band 10q21.3 and one a deletion of band 2q35.1. An abnormal FISH pattern was observed in 1/5 RARS, in 7/27 (25.9%) RA, in 3/5 RCMD, in 8/22 (36.4%) RAEB-1 and in 7/14 (50%) RAEB-2. Considering the IPSS, at least one defect was observed in 4/24 (16.6%) low-risk, in 12/28 (42.8%) intermediate-1 risk and in 7/15 (46.6%) intermediate-2 risk 3/6 (50%) high-risk patients. Disease evolution occurred in 2 RA patients, in 3 RAEB-1 and in 4 RAEB-2 patients with an abnormal FISH pattern. Seven of these patients presented at least two chromosomal deletions. In contrast, disease evolution occurred in one RARS, in two RA, 3 RAEB-1 and in 5 RAEB-2 with a normal FISH pattern. In conclusion our data suggest that FISH: i) reveals novel unsuspected chromosomal lesions, ii) in about 36% of chromosomally normal MDS patients, iii) these chromosomal lesions mostly consist in gains/losses, whereas balanced rearrangements are very rare; iv) an abnormal FISH pattern with more than two deletions seems to correlate with disease progression.

NF-KB REGULATES FAS GENE EXPRESSION IN MYELODYSPLASTIC SYNDROMES

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7Background. Overexpression of death receptor Fas and its ligand FasL, plays a major role in the activation of extrinsic pathway of apoptosis in MDS. TNF-a, a potent activator of the NFkB pathway, upregulates Fas in normal bone marrow cells controlled by NFkB and hepatocytes, while FasL is regulated by FOXO3A which nuclear translocation is inhibited by NFkB. Aims. This study aims at investigating the role of NFkB in the regulation of Fas and FasL in MDS/AML cells. Methods. Localization of p65 NFkB subunit by IF and WB on nuclear fractions, Fas expression by RT-PCR and Fas and FasL proteins by flow cytometry were performed in P39 and HL-60 cell lines and in bone marrow-derived MDS/AML mononuclear (n=11) or CD34+ cells (n=3) maintained in vitro by ChIP. NF-kB pathway was inhibited by ChIP. NF-κB pathway was implicated in the regulation of Fas expression in MDS/AML cells. Induction of Fas together with downregulation of FasL lead to the inhibition of Fas-dependent apoptosis, a mechanism by which NFκB may contribute to the progression of the disease.

MDS and other bone marrow failure syndromes - Clinical 1

EVALUATION OF DYSPLASTIC FEATURES IN MYELODYSPLASTIC SYNDROMES: PROPOSAL FOR A STANDARDIZED MORPHOLOGICAL PANEL

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Background. WHO proposal for myelodysplastic syndrome (MDS) classification introduced uni- versus multilineage dysplasia as a diagnostic criterion in MDS with <5% bone marrow (BM) blasts, increasing the prognostic value of this classification. Cytopenias generally correspond to dysplasia, but discordance may be present. However, a structured and reproducible approach for the precise recognition of BM dysplasia is still missing, and the relationship between cytopenia and dysplasia needs to be clarified. Aims. The aims of this study were to identify a panel of reproducible morphological criteria associated with MDS useful for a correct application of WHO classification and to evaluate the prognostic relevance of the single morphological abnormalities and of the total lineage dysplasia as well as the degree of dysplasia. Methods. We retrospectively examined the cytological features of BM smears from 429 MDS patients previously classified according to FAB criteria, 214 patients with hyporegenerative anemia and 74 healthy subjects. By counting 100 cells for the erythroid and granulocytic lineages and at least 20 megakaryocytes and classifying them for their dysplastic changes, a panel of dysplastic features showing a better sensitivity and specificity for MDS identification was developed. The morphological panel including 26 dysplastic features (12 erythroid, 8 granulocytic and 6 megakaryocytic) was employed, in association with the evaluation of blast and sideroblast percentages, to reclassify MDS patients by 2008 revised WHO proposal using the 10% threshold to record dysplasia in the erythroid and granulocytic lineages and the 25% threshold for dysmegakaryopoiesis, with a between-investigators and within-investigator agreement of 92% and 95% respectively. Results. Three hundred and one MDS cases were correctly reclassified, 45 were unclassifiable for inadequate BM smears and 83 belonged to other hematopoietic neoplasms. On univariate analysis, increase of BM blasts, multilineage dysplasia and two or more cytopenas were associated with worse outcome but multivariate analysis failed to confirm the prognostic value of cytopenias. In MDS without an increase of BM blasts, Kaplan Meier estimates of overall survival (OS) and leukemia-free survival (LFS) showed that all patients with multilineage dysplasia had a significantly worse outcome, independently of the number of cytopenias (P=0.008 and P=0.0005 respectively). Some morphological abnormalities, i.e. erythroblast irregular nuclear edges or multinucularity, granulocyte hypo-agranularity, small binucleated megakaryocytes, were associated with poor outcome, and total granulocytic or megakaryocytic dysplasia showed a significant independent unfavorable prognostic value (P=0.0004 and P=0.0001 respectively). Also the degree of granulocytic or megakaryocytic dysplasia, estimated based on percentage of dysplastic cells, was found to have a significant effect on OS (P=0.002 and P=0.0003 respectively). Conclusions. The definition of BM dysplasia with a standardized morphological panel that improves the objectivity and reproducibility of microscopic analysis is useful for a correct application of WHO classification as well as for the differential diagnosis between MDS and other cyto- penias. Our data confirm the significance of multilineage dysplasia correlation with high-grade MDS. Prognostic systems including the evaluation of the degree of BM dysplasia should be adopted for clinical decision-making and selection of MDS patients for new effective targeted therapies.
LONG TERM OUTCOMES IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) WITH SUSTAINED ECUILIZUMAB TREATMENT

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Figure 1. 10 Saint-Louis Hospital, Paris, France 9 Duke University Medical Center, Durham, United States of America 8 The Royal Melbourne Hospital, Parkville, Australia 7 Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands 3 University of Ulm Transfusion Medicine, Inst. for Clin Transfusion Med & Immunolog, Ulm, Germany 2 Evangelisches Krankenhaus Hamm, Hamm, Germany 1 Taussig Cancer Institute, Cleveland Clinic, Cleveland, United States of America 4 University Hospital Essen, Essen, Germany 1 St. James’s University Hospital, Leeds, United Kingdom

Background. PNH is a chronic, life-threatening disease associated with increased risk of thrombosis (TE), end organ damage, often poor quality of life, with premature mortality. TE accounts for 40–67% of PNH-related deaths; anticoagulation (AC) management may not be effective in PNH, as TE rates in AC-treated PNH patients remain elevated. The complement inhibitor eculizumab reduces chronic hemolysis rapidly and significantly; it also leads to reductions in TE events pulmonary hypertension and improvements in chronic kidney disease (CKD) and quality of life. Methods. All patients (N=195) in the PNH eculizumab clinical trials and subsequent Extension studies were evaluated for safety, sustained outcomes, and patient survival. Median age was 40 yrs, 54% female, 29% with history of aplastic anemia and 1.5% with history of myelodysplasia. TE was reported in 32% (63/195) of patients prior to eculizumab treatment. Results. Median eculizumab treatment duration was 29 mo (IQR: 16–55). Total eculizumab exposure was 467.1 patient-years. LDH was rapidly and significantly reduced from baseline of 2,293 U/L (~10×ULN) to 310 U/L post 1 month treatment (P<0.0001) and was sustained through 36 months (P<0.0001). TEs were significantly reduced from 52 pre-treatment to 10 trial events by matched-time analysis (P<0.0005). Of the 7/195 patients who experienced a TE, 5 had history of TE and 2 were concomitantly treated with AC. Of patients treated with AC, 59/98 experienced at least 1 TE prior to treatment. In 11 patients who discontinued AC while on eculizumab, no TEs were reported during or following AC discontinuation. Chronic Kidney Disease (CKD) was reduced from 69% at baseline to 51% (n=29) 36 months post-treatment. Significant increases in hemoglobin were sustained over 36 months treatment (mean increase over baseline at 36 months: 9.5 g/L; P<0.0001; range: -31.68, despite significant and sustained reductions in transfusion requirements. Of the 87/195 patients receiving at least 36 months of eculizumab, 29% (25/87) became transfusion independent and maintained transfusion independence for the entire treatment period. Eculizumab was well tolerated; 90% (175/195) of patients completed the parent and extension trials. Twenty patients (10%) did not complete the trial including 9 patients following a reported adverse event (AE). In 16 week follow-up, TE was reported in 3 of these 20 patients, including 1 death. Most AEs (91%) were mild or moderate in severity. There were 2 cases of meningococcal sepsis; both were successfully treated without sequelae. There were 4 patient deaths: 3 not related and 1 possibly related to eculizumab, per investigator. Kaplan-Meier analysis (fig.1) showed probability of overall survival was 97.64% at 3 years and was maintained through 5.5 years of ongoing treatment. Patient survival compares favorably to a predicted survival rate previously reported in historical controls of 65% at 5 years. Conclusion. Long term reduction of chronic hemolysis in PNH patients treated with eculizumab is associated with significant improvements in the incidence of TE, CKD, and other PNH-associated symptoms. Long term treatment with eculizumab also results in a high probability of survival, which is maintained over 5.5 years of ongoing treatment.

ALEMTUZUMAB FOR APLASTIC ANEMIA AND RELATED IMMUNE-MEDIATED BONE MARROW FAILURES: LONG-TERM FOLLOW UP OF A PILOT STUDY

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Background. Immunosuppression is a worthy treatment option for patients suffering from aplastic anemia (AA) or other lineage-specific immune-mediated marrow failure, such as pure red or white cell aplasia (PRCA and PWCA). We previously described results from a pilot phase II prospective trial (NCT00895739) investigating the anti-CD52 monoclonal antibody alemtuzumab in combination with low-dose cyclosporine A (CyA; Risitano, BJH 2010;148:791). Here we report the long-term follow up of the study. Methods. Twenty-eight patients were enrolled in the study: 13 SAA, 13 PRCA and 2 PWCA. Of the 87/195 patients receiving at least 36 months of eculizumab treatment duration was 29 mo (1-66;IQR:23-32m); total eculizumab exposure was 467.1 patient-years. On average, patients received at least 1 TE per year (range: 0–5), with 5 TEs per patient in 11 patients. Total TEs were significantly reduced from 52 pre-treatment to 10 trial events by matched-time analysis (P<0.0005). Of the 7/195 patients who experienced a TE, 5 had history of TE and 2 were concomitantly treated with AC. Of patients treated with AC, 59/98 experienced at least 1 TE prior to treatment. In 11 patients who discontinued AC while on eculizumab, no TEs were reported during or following AC discontinuation. Chronic Kidney Disease (CKD) was reduced from 69% at baseline to 51% (n=29) 36 months post-treatment. Significant increases in hemoglobin were sustained over 36 months treatment (mean increase over baseline at 36 months: 9.5 g/L; P<0.0001; range: -31.68, despite significant and sustained reductions in transfusion requirements. Of the 87/195 patients receiving at least 36 months of eculizumab, 29% (25/87) became transfusion independent and maintained transfusion independence for the entire treatment period. Eculizumab was well tolerated; 90% (175/195) of patients completed the parent and extension trials. Twenty patients (10%) did not complete the trial including 9 patients following a reported adverse event (AE). In 16 week follow-up, TE was reported in 3 of these 20 patients, including 1 death. Most AEs (91%) were mild or moderate in severity. There were 2 cases of meningococcal sepsis; both were successfully treated without sequelae. There were 4 patient deaths: 3 not related and 1 possibly related to eculizumab, per investigator. Kaplan-Meier analysis (fig.1) showed probability of overall survival was 97.64% at 3 years and was maintained through 5.5 years of ongoing treatment. Patient survival compares favorably to a predicted survival rate previously reported in historical controls of 65% at 5 years. Conclusion. Long term reduction of chronic hemolysis in PNH patients treated with eculizumab is associated with significant improvements in the incidence of TE, CKD, and other PNH-associated symptoms. Long term treatment with eculizumab also results in a high probability of survival, which is maintained over 5.5 years of ongoing treatment.

Figure 1.
PROGNOSTIC VALUE OF IRON PARAMETERS AT DIAGNOSIS IN MYELODYSPLASTIC SYNDROME PATIENTS: ANALYSIS OF 643 PATIENTS FROM THE PIEDMONT MDS REGISTRY

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Background. The role of serum ferritin (SF) value as prognostic factor in patients affected by myelodysplastic syndrome (MDS) is still controversial. SF levels mainly due to transfusion requirement has been associated with lower overall survival (OS) (Malcovati, 2007) and increased risk of leukemic transformation (Sanz, ASH 2008; de Swart ASH 2010). Conversely, Park and colleagues (ASH 2010) failed to identify a negative prognostic value of SF higher than 300 ng/mL at baseline in a cohort of low risk untransfused MDS patients. Little is known about the impact on survival of other iron parameters such as transferrin saturation (TS). Aims. We evaluated the prognostic significance of SF and TS at diagnosis in MDS patients analyzing data collected in the MDS Piedmont Registry. Methods. Data from 1558 patients from 37 centers included from 1999 to 2010 were analyzed. We evaluated OS according to the following parameters: FAB classification (M0-2, M6), WHO classification (M0-2, M6), IPSS and number of cytopenias (Table 1). This study was approved by the National Ethical Committee Board, with the signed informed consent of all patients. Conclusion. SF >500 ng/mL also in the subgroup of non TD patients. Conversely, increased SF levels mainly due to transfusion requirement has been associated with lower overall survival (OS) (Malcovati, 2007) and increased risk of leukemic transformation (Sanz, ASH 2008; de Swart ASH 2010). Increased SF levels mainly due to transfusion requirement has been associated with lower overall survival (OS) (Malcovati, 2007) and increased risk of leukemic transformation (Sanz, ASH 2008; de Swart ASH 2010). Increased SF levels mainly due to transfusion requirement has been associated with lower overall survival (OS) (Malcovati, 2007) and increased risk of leukemic transformation (Sanz, ASH 2008; de Swart ASH 2010).
the number of cytopenias (although not statistically significant). Conclusions. During the early stages of MDS, one mechanism contributing to hypopcellular marrow and peripheral blood cytopenia is the significant increase of apoptosis in haematopoietic cells. The higher expression of FMNL1 in MDS CD3+ lymphocytes and bone marrow cells may be related to clonal or oligo-clonal T cell activation, since FMNL1 is important for the cytotoxic function of these cells. The CD4:CD8 imbalance could reflect an alteration in the immune regulation, which could contribute to the cytopenia in some MDS patients. Further studies are required to test these hypotheses. Supported by FAPESP, CNPq and INCT do Sangue.

0259
THE PRESENCE OF ABERRANT MYELOID PROGENITORS PREDICTS OVERALL SURVIVAL IN INTERMEDIATE-2 AND HIGH RISK MYELODYSPLASTIC SYNDROMES UPON TREATMENT WITH AZACITIDINE

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One of the major challenges in MDS is the prognostication and selection of the most suitable therapeutic options. Flow cytometric analysis of bone marrow cells in low and int-1 risk MDS patients can identify distinct subgroups within validated risk groups and predict response to growth factor treatment. (Westers et al., Blood 2010) In this study, we investigate the predictive value of flow cytometry (FC) for prognosis and selection of int-2 and high risk MDS patients for response to azacitidine. Bone marrow aspirates were analyzed by FC in 25 constitutive MDS patients before treatment and after every third cycle of azacitidine. A flow score was calculated using the flow cytometric scoring system (FCSS; Wells et al., Blood 2003). The FCSS is a scoring system that allows for a numerical display of immunophenotypic aberrancies in (im)mature myelo-monocytic cells (0-1 no flow cytometric dysplasia, 2-3 mild dysplasia, 4 severe dysplasia); e.g. high scores (≥4) reflect a high number of aberrancies. Response was evaluated using IWG-2006 criteria. The WHO 2008 categories were 2 RCMDS, 5 RAEB-1, 9 RAEB-2, 7 AML with multilineage dysplasia and 6 CMML. Flow cytometric follow up was available in 17 patients. Median follow up time after initiation of the first cycle was 9.6 months. Four patients achieved complete remission (CR), 7 stable disease (SD) and 6 had progressive disease (PD). The median FCSS at baseline was 7 (range: 2-8). Patients who achieved CR showed a significant decrease in the FCSS after 5 cycles as compared to patients with SD and PD (median FCSS 1.5, 5 (p=0.009) and 6.5 (p=0.003), respectively). At baseline, 16/25 patients had aberrant marker expression (AME) (i.e. CD5, CD7, CD11b and/or CD56) on myeloid progenitors and/or monocytes. These patients had significantly worse overall survival as compared to patients without AME (p=0.004; relative risk (RR) of death in patients with AME was 6 times higher than without AME). Absence of AME in patients with SD was strongly associated with erythroid response (HI-E). Our data indicate that patients with SD and AME are less likely to show HI-E upon azacitidine treatment (RR=4). In conclusion, persistent high FCSS during treatment and/or presence of AME at baseline is of prognostic value and identifies int-2 and high risk MDS patients who are unlikely to achieve CR or HI-E and with worse overall survival as compared to patients without AME.

0260
CLINICAL CHARACTERISTICS OF THERAPY-RELATED MYELODYSPLASTIC SYNDROME

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We identified 483 patients who have had one or more malignancies prior to the diagnosis of MDS evaluated at MDACC between 1997 and 2007. To select therapy-related myelodysplastic syndrome (Tx-MDS), we removed patients who have diagnosed as MDS by FAB classification but have not included in WHO classification, and we selected patients who have undergone chemotherapy (CTx) and/or radiation therapy (RTx) for prior malignancies (Fig 1). Finally 281 patients were included for the analysis of Tx-MDS. Male sex was 165 (58.7%) and median age at diagnosis of MDS was 65.2 years (range 13.4-89.4). Patients were classified by WHO classification as follows: RA (n=63, 22.4%), RARS (n=29, 10.3%), RCMD/RCMD-RS (22, 7.9%), RAEB-1 (n=90, 32.0%), RAEB-2 (n=67, 23.8%), MDSu (n=10, 3.6%). IPSS was low in 30 (11%) patients, INT-1 in 87 (31.0%) patients, INT-2 in 120 (42.7%) patients and high in 35 (12.5%) patients. The most common cytogenetic abnormality was -5 and/or -7 (n=149, 53.1%). Seventy five patients (26.7%) were diploid. Prior cancers included: head and neck (n=7, 2.5%), thyroid (n=3, 1.1%), lung (n=7, 2.5%), breast (n=32, 11.4%), gastrointestinal (n=13, 4.6%), prostate (n=54, 12.1%), other germinatary or gynecological (n=16, 5.7%), melanoma/skin cancers (n=5, 1.8%), sarcomas (n=8, 2.8%), other solid cancers (n=2, 0.7%), lymphoma (n=102, 36.3%), CML/CLL (n=6, 2.1%), AML/ALL (n=5, 1.8%), multiple myeloma (n=11, 3.9%) and multiple cancers (n=30, 10.7%). Prior Tx was CTx only (n=107, 38.1%), RTx only (n=73, 26.0%) or both CTx and RTx (n=101, 35.9%). The treatments of Tx-MDS were categorized as follows: supportive care/cytokine therapy in 126 (44.8%); non-cytotoxic drugs in 77 (27.4%); cytotoxic chemotherapy in 65 (23.1%); hematopoietic stem cell transplantation (HSCT) in 15 (4.6%). A total of 54 patients had received HSCT (autologous, n=52 (18.5%); allogeneic, n=2 (0.7%)). Univariate analyses for survival revealed that presence of hepatomeglay (no hepatomeglay vs. hepatomeglay; p=0.023), cytogenetics (6-, 20q-, Y-; normal vs. others; p<0.001), types of MDS by WHO classification (RA, RCMD, MDSu vs. FAB classification: RAEB-1, RAEB-2, MDSu) and a diagnosis of MDS more than 5 years after the diagnosis of cancer were associated with a worse overall survival. Multivariate analysis confirmed the above findings. Overall survival (OS) was significantly shorter in patients who achieved a complete response (n=16, 5.6%) than in patients who did not achieve a complete response (n=265, 94.4%) (p<0.001; RR=3.35). OS was significantly worse for patients with a high IPSS score (p=0.04; RR=1.75). Median OS was 17 months in patients with clonal abnormalities compared to 35 months in patients without (p=0.01; RR=3.45). When the whole group of patients was categorized into groups according to the number of prior malignancies, patients who had had more than one prior malignancy had a shorter OS compared to patients with one previous malignancy (p=0.04; RR=2.35). In conclusion, the number and type of prior malignancies, presence of clonal abnormalities and a high IPSS score are significantly associated with worse overall survival in patients with a history of a prior malignancy who have developed a secondary MDS.

Figure 1. Overall survival by chromosomal abnormalities.
others; \( p<0.001 \)), time from \( T_x \) to MDS \((≤5 \text{ vs. } >5Y; p=0.027) \), number of line(s) of therapy \((1 \text{ vs. } ≥2; p=0.011) \), serum albumin \((≥4 \text{ vs. } <4\text{g/dL}; p=0.065) \), serum beta2 microglobulin \((≥3 \text{ vs. } >3\text{mg/L}; p=0.015) \), serum creatinine \((≥1 \text{ vs. } >1\text{mg/dL}; p=0.061) \), ECOG performance status \((0-1 \text{ vs. } ≥2; p=0.001) \) as significant. Age \((≥65 \text{ vs. } >65; p=0.109) \), sex \((\text{male vs. female}; p=0.862) \), prior \( T_x \) \((C/Tx \text{ vs. RTx only}; p=0.471) \), prior malignancies \((\text{hematological vs. solid cancer}; p=0.650) \), prior lymphoma \((\text{lymphoma vs. non-lymphoma}; p=0.055) \), prior HSCT \((\text{ASCT vs. alloHSCT vs. none}; p=0.691) \) and serum ferritin level \((≥600 \text{ vs. } >600\text{ng/mL}; p=0.420) \) were not significant. The events of leukemic evolution were not consistent with the risk groups: 3 \((10.1\%) \) in low, 12 \((13.5\%) \) in INT-1, 9 \((8.3\%) \) in INT-2 and 9 \((25.0\%) \) in high by IPSS. High risk group in IPSS showed high possibility of leukemic evolution \((p=0.039) \).

0261
CYTOGENETIC CHARACTERISTIC OF MYELODYSPLASTIC SYNDROME IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED PATIENTS; HIGH INCIDENCE OF POOR PROGNOSTIC KARYOTYPE AND CHROMOSOME 7 ABNORMALITIES
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Background: Human Immunodeficiency Virus (HIV) infection can often cause myelodysplastic features in bone marrow. However bona fide myelodysplastic syndrome (MDS) is not common in this population. Data are lacking whether HIV associated MDS has distinct clinical features from non-HIV MDS. Aims: The aim of this study was to compare the clinical characteristics of HIV associated MDS and non-HIV MDS. Methods: This is the retrospective cohort study with 91 patients who were diagnosed with MDS in our allied three teaching hospitals in New York City from 2005 to 2010. Their clinical history, pathological data, cytogenetic studies, and laboratory data were obtained through electronic medical records. HIV diagnosis was confirmed based on serological testing of enzyme-linked immunosorbent assay (ELISA) with Western Blot confirmation as well as HIV-1/2 viral PCR. MDS diagnosis was confirmed with bone marrow morphology, laboratory data and cytogenetic studies and was classified both according to the French-American-British (FAB) classification and World Health Organization (WHO) criteria. Research protocol was approved by our Institutional Review Board. Results: Within the cohort of 91 patients with MDS, 9 patients carried diagnosis of HIV and 82 patients were non-HIV. Karyotype abnormalities were more associated with HIV related MDS \((88\% \text{ vs. } 39\% \ p<0.01) \). Additionally, poor prognostic karyotype abnormalities were more associated with HIV related MDS according to the International Prognostic Scoring System (IPSS) \((66\% \text{ vs. } 16\%; p<0.01) \). Number 7 chromosome abnormalities which are also considered to be poor prognostic marker, was highly involved in HIV related MDS cohort \((77\% \text{ vs. } 15.6\% \ p<0.01) \). Within the cases of HIV related MDS, 8 out of 9 patients had a follow up data. Their median survival after diagnosis of MDS was 9.3 months and rate of transformation to acute leukemia was 62.5\% \((n=5) \). Median survival after transformation was 3.6 months. Conclusions: HIV related MDS has higher incidence of poor prognostic karyotype abnormalities compared to non-HIV MDS cohort and has high rate of transformation and poor survival. This result suggests that HIV infection itself, antiretroviral therapy, or immunodeficiency state might be associated with clonal mutagenesis in bone marrow.

0262
THE QUANTITY OF P15INK4B METHYLATION IS LOWER IN PEDIATRIC MDS THAN IN ADULTS, BUT CORRELATES WITH BONE MARROW BLAST PERCENTAGE AND SURVIVAL
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Background: Inactivation by promoter hypermethylation of \(p15INK4b\) is believed to contribute to the initiation and progression of MDS in adults. Contrary to demonstrated benefits of demethylating agent in adults, the use of demethylating agent has not been reported for childhood MDS. Aims: To evaluate the potential of the use of demethylating agent in childhood MDS, we performed a quantitative analysis on \(p15INK4b\) promoter methylation. Methods: The study included 41 childhood MDS (22 RCC, 14 RAEB and 5 JMML) and 12 healthy bone marrow donors as controls. Pyrosequencing was performed using PSQ96MA system (Biotage, Uppsala, Sweden). The mean \% of methylated cytosine (methylation level: MtL) in childhood MDS was compared with that of adult MDS in the previous study. Results: The MtL of \(p15INK4b\) was lower in children than in adults both in control group \((1.62\% \text{ vs. } 4.30\% \ p=0.001) \) and in MDS group \((2.57\% \text{ vs. } 8.76\% \ p=0.001) \). The childhood MDS patients with \(>5\% \) BM blasts showed higher MtL than those with \(<5\% \) BM blasts \((3.28\% \text{ vs. } 1.92\% \ p=0.098) \). MtL >2.50\% was a poor prognostic factor in childhood MDS \((p=0.075, \text{ univariate analysis}) \). Conclusions: To our knowledge, this is the first study which quantitatively analyzed \(p15INK4b\) methylation in childhood MDS. In conclusion, the methylation quantity of \(p15INK4b\) of children is lower than that of adults both in control and in MDS patients. The methylation quantity of \(p15INK4b\) of childhood MDS is higher than that of normals. The high methylation quantity of \(p15INK4b\) is associated with BM blasts and shorter mean survival in childhood MDS.

0263
COMPARABLE OUTCOME OF IMMUNOSUPPRESSIVE THERAPY WITH RABBIT ANTI-THYMOCYTE GLOBULIN (ATG) PLUS CYCLOSPORIN A (CSA) TO ONE WITH HORSE ATG PLUS CSA IN REFRACTORY CYTOPENIA IN CHILDHOOD
A Yoshihime-Nollke,\(^1\) M van den Heuvel-Eibrink,\(^1\) I Baumann,\(^1\) M Führer,\(^2\) B Moerloose,\(^3\) M Dvorzak,\(^4\) J Star,\(^7\) M Schmugge,\(^8\) M Zecca,\(^9\) P Nollke,\(^2\) A Fischer,\(^1\) F Locatelli,\(^1\) B Strahm,\(^1\) C Niemeyer\(^2\)
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Background. Refractory cytopenia in childhood (RCC) is the most common subtype of pediatric myelodysplastic syndrome (MDS). In
addition to allogeneic hematopoietic stem cell transplantation (HSCT). Immunosuppressive therapy (IST) has recently been introduced as a treatment modality. Annals. Because horse anti-thymocyte globulin (h-ATG) is currently not available in Europe, rabbit ATG (r-ATG) is widely used. However, the information on the efficacy of rabbit ATG in IST is limited. Here we report the results of IST with ATG and cytogenetic analysis (CSA) in patients (pts) with RCC, comparing r-ATG and h-ATG. Patients. Ninety-one pts (52 boys/59 girls) with RCC were given IST as first line treatment between 1999 and 2009. At diagnosis, the median age was 9.8 (1.2-18.1) years. The median absolute neutrophil count (ANC) was 315 (0-3038) x10^9/L. 77 pts were transplants dependent for platelets and 66 for red cells. Bone marrow cellularity was low in 78 pts and normo/hypercellular in 8 pts. Cytogenetic analysis revealed a normal karyotype in 49 pts, an abnormal clone in 3 pts and no result in 39 pts. IST was started at a median of 67 (0-472) days after diagnosis. Fifty-four pts was given Thymoglobulin® (r-ATG group), 34 pts Lymphoglobulin® (h-ATG group), and 4 pts others. There were no statistical differences in patient characteristics listed above between r-ATG and h-ATG groups. Since 2007 h-ATG has been replaced by r-ATG. Results. 57 (65 %) pts responded to IST at 6 months (complete response =normal blood count (CR): n=6, partial response (PR): n=51), with no difference between r-ATG and h-ATG (57.6% vs. 68.5%, respectively, p=n.s.). There were also no differences in response rate according to age, days between diagnosis and IST, and ANC at IST. Clonal evolution occurred in 6 pts (5 pts in r-ATG-, 1 pt in h-ATG-groups; cumulative incidence at 5 years =10.2%), 3/6 pts developed -7 or 7q- aberrations Seven responders experienced recurrent disease (2 pts: r-ATG, 5 pt: h-ATG groups). One child (h-ATG) developed de novo PNH. Thirty-five pts received HSCT as the second line therapy. The overall survivals (OS) were 85.1% in r-ATG group (at 3 yrs) and 91.2% in h-ATG group (at 5 yrs), and the failure free survivals (FFS) were 41.2% in r-ATG group (at 5 yrs) and 43.6 in h-ATG group (at 5 yrs) (p=n.s.), respectively. The median follow-up after ATG was 510 days (105-1095) in r-ATG group and 1476 days (143672) in h-ATG group. Summary. In this selected patient population with RCC, about 60% of pts responded to IST. This finding suggests that the immune system plays the key role in the pathophysiology of bone marrow failure in some pts with RCC. There was no difference in response rate between r-ATG and h-ATG groups. Although the follow-up time is short in the r-ATG group, there were also no differences in OS and FFS between 2 groups. These results justify the further application of r-ATG in the treatment of RCC.

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THE QUANTITY OF CLONAL CELLS DETECTED BY CONVENTIONAL CYTOGENETIC ANALYSIS CORRELATES WITH BONE MARROW BLASTS AND LEUKEMIA FREE SURVIVAL IN MYELODYSPLASTIC SYNDROMES
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Backgrounds. Most researches studied clonal chromosomal abnormalities in the relationship between the presence or absence of abnormality of certain chromosome and the prognosis of MDS. However, little has been known about the utility of the quantitative result of clonality studies in clinical practice. Annals. This study investigated the novel roles of CCA and FISH results in predicting outcome of MDS patients. The results of CCA and FISH were compared both in qualitative and in quantitative aspects. We analyzed the prognostic significance of clonal cells in CCA or FISH in MDS. Methods. We performed the quantitative and qualitative analyses of conventional cytogenetic analysis (CCA) and interphase FISH (iFISH) results in 129 MDS patients, and investigated their unknown roles in predicting prognosis. Results. The abnormalities of -5q, -7q, -8, -20q and +1q were detected in 15.2%, 14.0%, 19.4%, 7.0% and 7.8%, respectively. iFISH detected occult abnormalities in 18.6% (24/129), changing IPSS grouping in 4.9% (6/129). The proportion of clonal cells for each chromosome of CCA did not correlate with the result of iFISH (Δ, from 0.580 to 0.775). The clonal cell percentage in CCA was related to patients with >5% bone marrow blasts than those with <5% (57.4% vs. 24.4%, p=0.066). Multivariate analysis showed high proportion of clonal cells in CCA analysis is an independent prognostic factor for progression free survival into AML in MDS (p=0.059). Conclusions. iFISH is advantageous in identifying cryptic cytogenetic abnormalities and can change the IPSS risk grouping. We showed the quantity of clonal cells detected in CCA correlates with the bone marrow blast percentage, and the progression free survival into AML, suggesting the novel diagnostic utility of CCA in MDS.
respectively (p=0.04 for RTC vs. others). Multivariate analysis identified RTC as a favorable prognostic factor. HR 0.4 (0.2-0.9, p=0.04). Age, gender, donor type, IPSS, blast excess and cytogenetics were not predictive. MAC and unrelated donor were predicting factors for NRM with HR 3.0 (1.2-7.5, p=0.02). ACE predicted for NRM with HR 2.0 (1.2-3.2, p=0.005). RIC predicted for NRM with HR 2.1 (1.2-3.6, p=0.009). Median follow-up for NRM was 7.0 years (range 0.1-17 years). Median follow-up for transplant related mortality was 7.0 years (range 0.1-16 years).

Conclusions. Hypocellular MDS had similar clinical features and similar survival compared to normo/hypercellular MDS. However, hypocellular MDS patients had a different pattern from those of normo/hypercellular MDS regarding prognostic factors.

A 5-DAY OUTPATIENT REGIMEN OF 5AZACITIDINE IS WELL-TOLERATED AND EFFECTIVE FOR HIGH-RISK MYELODYSPLASTIC SYNDROME AND ACUTE MYELOID LEUKEMIA PATIENTS UNSUITABLE FOR AGGRESSIVE CHEMOTHERAPY

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Background. Azacitidine has been established as an effective agent for patients with myelodysplastic syndrome (MDS), with prolongation of survival and delayed progression to Acute Myeloid Leukemia (AML). Patients with AML who are unsuitable for aggressive therapy or those with refractory disease may also benefit from therapy with this agent. Azacitidine is usually delivered according to a 7-day regimen, which may be difficult to deliver in the day ward setting. Aims. To examine the use of azacitidine delivered as a five-day regimen in patients with very high risk MDS and AML patients not suitable for intensive chemotherapy (off-label use of azacitidine). Methods. We report the use of a 5-day regimen of azacitidine in 70 patients with MDS (n=54) or AML (n=26) in 4 centres in Ireland. Patients received azacitidine 75mg or 100mg/m²/day subcutaneously or intravenously for 5 days in a day ward setting. Cycles were given at 22-day intervals and continued until disease progression. Results. Of the 70 patients treated 48 were male and 22 female. Median age at time of treatment was 71 years (range 36-89 years). MDS patients included 14 with RAEB-2, 9 with RAEB-1, and 7 with CMML. Others included RARS (n=2), RCMD (n=2), RAEB (n=2), and 1 with MDS and AML. AML patients included 18 with de novo AML and 18 with AML secondary to MDS (data not available for 5 patients). Most patients had received prior intensive AML induction chemotherapy but were unsuitable for further intensive therapy due to age, infection or comorbidities, had primary refractory AML or relapsed AML. Eight patients received azacitidine as primary therapy for AML. Median survival in this very poor prognosis AML group was 5 months (range 1-14 months), 50% had stable disease during treatment. Of 22 patients who had >4 cycles of therapy, median survival was 10 months (range 1-35 months). Of 7 patients with CMML, 4 patients had prolonged survival >20 months. AML patients including 18 with de novo AML and 18 with AML secondary to MDS had a median survival of 5 months (range 1-14 months), 50% had stable disease during treatment. Of 22 patients who had >4 cycles of therapy, median survival was 10 months (range 5-14 months). Conclusions. Azacitidine was well-tolerated and effective in both MDS and AML patients, with many remaining transfusion independent. In the poor-prognosis AML group, there was significant reduction in in-patient admissions, compared to the use of intensive chemotherapy. A 5-day regimen of azacitidine, conveniently delivered in the day ward or outpatient setting, is effective in high-risk MDS and offers a well-tolerated palliative out-patient therapy for patients with very poor prognosis AML.

CLINICAL AND BIOLOGICAL ROLE OF VASCULAR ENDOTHelial GROWTH FACTOR AND ITS RECEPTORS IN MYELODYSPLASTIC SYNDROMES

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Background. The angiogenesis mediators are altered in myelodysplastic syndromes (MDS) and abnormal angiogenesis is implicated in
the pathogenesis of these disorders. VEGF is the most important proangiogenic factor and exerts its biologic effects by interaction with its specific receptors i.e. VEGF-R1 and VEGF-R2. The VEGF/receptor signaling system is involved in the regulation of angiogenesis and hematopoiesis, the former through a paracrine loop and the latter through an autocrine loop. Aims: We analyzed bone marrow (BM) immunohistochemical expression of VEGF-Rs and VEGF in MDS patients. Furthermore, we investigated if these parameters had an impact on patient clinical outcome, in order to define their potential prognostic value. Methods: Study population included 79 MDS patients, categorized according to WHO 2008 classification and stratified in prognostic categories by means of IPSS and the WPSS systems, and 20 age-matched normal controls (NC). Bone marrow VEGF (and VEGF-Rs) expression was correlated to BM cellularity through an index (i): [(% of BM cellularity x %VEGF-positive cells)/104]). Results: VEGF had a weak cytoplasmic expression in BM pro-erythroblasts, whereas normoblasts were not immunoreactive. In the myeloid lineage, VEGF expression was more intense in immature cells. VEGFi was higher in MDS in comparison to NCs (Mann-Whitney U-test, p=.006) and was even distributed among the different IPSS and WPSS categories. VEGF-R1 had a moderate cytoplasmic expression in myeloid precursors, at all stages of differentiation. VEGF-R1i was higher in MDSs than in NCs (Mann-Whitney U-test, p=.08). VEGF-R1i significantly differed among IPSS and WPSS prognostic classes (Kruskal-Wallis analysis, p=.04 and p=.003). VEGF-R2i was expressed in myeloid precursors with a moderate cytoplasmic positivity. Myeloid mature cells were not immunoreactive. There were no difference in VEGF-R2i in MDSs as compared to NCs. In MDSs, both VEGF-R1i and VEGF-R2i directly correlated with VEGF-R1 and VEGF-R2i expression (Spearman test, r=64; p<.0001 and r=.47, p<.0001, respectively). Considering the 75th percentiles of VEGFi and VEGF-R1i values, Low and High VEGFi and VEGF-R1i classes could be determined. Low-VEGF patients had a longer LFS (Kaplan-Meyer, p=.006) and a significant better OS (Kaplan-Meyer, p=.05) compared with High-VEGF. Similarly, Low-VEGF-R1 had a longer LFS (Kaplan-Meyer, p=.001) and a better OS (Kaplan-Meyer, p<.001), compared to High-VEGF-R1 patients. Nevertheless, in a multivariable analysis stratified by IPSS, VEGFi and VEGF-R1i did not retain a significant effect on both OS and LFS. No significant correlation between VEGF-R1i and clinical variables was found. Summary/Conclusions: The enhanced cytoplasmic expression of both VEGF-R1i and VEGF-R2i in BM of MDS patients and their prognostic impact on LFS and OS, although in univariate analysis, may suggest the hypothesis of an intracrine loop that may provide a growth advantage to neoplastic cells. Further studies, integrated with molecular approaches, will be needed to verify the role and the alterations of the VEGF/VEGF-receptors pathway in these neoplasms.

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FACTORS ASSOCIATED WITH HAEMATOLOGIC RESPONSES IN MYELODYSPLASTIC SYNDROMES (MDS) PATIENTS TREATED WITH DEFERASIROX: AN EPIC POST-HOC ANALYSIS USING INTERNATIONAL WORKING GROUP (IWG) 2006 CRITERIA

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Background. Reports of haematologic responses associated with iron chelation therapy in transfusion-dependent patients with MDS are emerging, although the mechanism by which these occur has yet to be elucidated. Not all patients have shown such haematologic improvement; hence it is important to determine factors that may be associated with this response. Aims: To evaluate change in serum ferritin and labile plasma iron (LPI) as potential predictors of haematologic responses to deferasirox in a post-hoc analysis of transfusion-dependent MDS patients enrolled in the 1-year prospective EPIC study. Methods.

Details of study design and inclusion/exclusion criteria for EPIC have previously been described (Gatterman et al, Leuk Res 2010). Recommended initial deferasirox dose was 20 mg/kg/day with dose adjustments of 5-10 mg/kg/day up to 40 mg/kg/day. MDS patients with haemoglobin (Hb) <11 g/dL or red blood cell (RBC) transfusion requirements >4 units/8 weeks and not receiving erythropoietin were eligible for erythroid response analysis. Patients with platelet counts <100 x 109/L or platelet-transfusion dependence, and absolute neutrophil counts <1.0 x 109/L and not receiving granulocyte-colony-stimulating factor or granulocyte macrophage colony-stimulating factor were selected for the assessment of platelet and neutrophil responses, respectively. Erythroid response: Hb increase ≥1.5 g/dL or reduction in transfusion requirements of ≥4 RBC transfusions/8 weeks. Platelet response: increase ≥50 x 109/L for patients with ≥20 x 109/L platelets or increase from <20 x 109/L to ≥20 x 109/L and by at least 100%. Neutrophil response: ≥100% increase and absolute increase ≥0.5 x 109/L. All responses to last ≥8 weeks (IWG 2006 criteria [Cheison et al. Blood 2006]). Changes in serum ferritin and LPI over time were assessed for haematologic responders and non-responders. Results. 279, 121 and 56 patients were included in erythroid, platelet and neutrophil response analyses, respectively. Erythroid responses were observed in 22.6% (63/279) of patients. Median time to response was 109 days. Platelet and neutrophil responses were observed in 14.0% (17/121) of patients after a median of 169 days and 19.6% (11/56) of patients after a median of 226 days, respectively. Median baseline serum ferritin levels were comparable among responders and non-responders (erythroid analysis: 5129 vs 2679 ng/mL; platelet analysis: 3228 vs 3383 ng/mL; neutrophil analysis: 2946 vs 3043 ng/mL). Median absolute change in
serum ferritin from baseline was greater in the haematologic responders compared with non-responders at end of study [Figure (A)] and at the time of haematologic response [Figure (B)], but the differences were not statistically significant. Mean pre-administration LPI levels were high at baseline (>0.4 µmol/L), but were reduced to below this threshold with deferasirox from week 12 onwards; the extent of LPI change did not differ between haematologic responders and non-responders (data not shown). Deferasirox is associated with improvements in haematologic parameters in some MDS patients. Change in serum ferritin was more pronounced in haematologic responders, suggesting that the serum ferritin decrease could play a role. However LPI did not appear to be related to the haematologic response seen with deferasirox. Additional factors influencing hematologic responses and the mechanisms behind these responses need further investigation.

0270
PRELIMINARY RESULTS OF A MULTICENTER RETROSPECTIVE STUDY ON EFFICACY AND SAFETY IN ERYTHROID STIMULATING AGENTS (ESAs) TREATMENT IN MYELOIDOSPLASTIC SYNDROME (MDS)

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Introduction. MDS is a group of clonal hematopoietic disorders characterized by ineffective hematopoiesis and frequent evolution to acute leukemia. ESAs in LR MDS moreover in HR MDS ESAs are able to obtain a response in about 50% of patients. Prospective studies are needed to validate these retrospective results.

0271
RENAL IMPAIRMENT IS A RISK FACTOR FOR EARLY MORTALITY IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Abstract. PNH is a chronic and progressive disease characterized by chronic complement mediated hemolysis leading to significant ischemic complications, end organ damage and shortened lifespan. It has been reported that renal failure accounts for 8-18% of PNH related deaths. The proposed complement mediated mechanism is multifactorial that includes development of renal-microthombi, ischemia, hemosiderin deposition due to high levels of free hemoglobin, and vasoconstriction of renal arteries reducing eGFR. Aim. To understand the impact of late stage renal impairment in patients with PNH defined as history of acute renal failure or reported eGFR<60 ml/min/1.73m2, we retrospectively analyzed medical charts of 301 PNH patients from national data registry in South-Korea over the last 41 years. Results. Patient ages ranged from 8 to 88 years (median 57 years), median PNH duration was 7.6 years (1 month to 41 years), and median PNH granulocyte clone size was 49% and median LDH was 4 fold above normal. Approximately 16% (50/301) of patients had a history or presence of late stage renal impairment, similar to the reported 20.5% of PNH patients with CKD 3-5 in the eculizumab clinical study (N=195). Median age of patients with late stage renal impairment was 38 years, and median granulocyte clone size was 34%. Patients with renal impairment accounted for 55% of patient deaths. Using a multivariate regression analysis renal impairment analysis was a strong predictor of mortality (p<0.0001; odds ratio 3.1; 95% CI 1.15 - 8.18). Patients with renal impairment had a significantly worse overall survival with a hazard ratio of 1.35 (95% CI 1.21-1.55; p<0.0001). Conclusion. Late stage renal impairment in patients with PNH is underestimated. These data establish renal impairment as a risk factor for early mortality in PNH patients.

0272
TREATMENT OF INTERMEDIATE AND HIGH RISK MYELOIDOSPLASTIC SYNDROMES(MDS) WITH AZACYTIDINE. THE HELLENIC EXPERIENCE

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Introduction. Myelodysplastic syndromes constitute an heterogeneous group of clonal hematopoietic disorders characterized by ineffective hematopoiesis and frequent evolution to acute leukemia. Azacytidine is a hypomethylating agent with significant efficacy and already approved for the treatment of MDS. Aim. The aim of this retrospective analysis was the investigation of the efficacy of azacytidine in a group of patients with intermediate and high risk MDS. Methods. 114 patients (57 female; 77 male) with a median age of 70 years (47-85) were included, azacytidine was administered at the dose of 75mg/m2 SC for 7 days every 28 days. Response to treatment was evaluated according to the International Working Group (IWG) criteria for MDS. Patients were...
classified according to FAB as follows RA n=15, RAEB n=67, RAEB-t n=16, AML n=6, RARS n=5, CMML n=5 and according to WHO as: RA n=3, RCMD n=8, RCMD-RS n=8, RARS n=2, RAEB-1 n=32, RAEB-II n=44, AML n=17. The IPSS was int-1 n=38, int-2 n=48, high n=26 and the WPSS, low n=5, intermediate n=14, high n=63, very high n=24. 65% of the patients were previously treated. 68% of the patients were RBC and 19% PLT transfusion dependent. Results. The median time to therapy initiation since diagnosis was 8 months (0-114), while the median number of cycles administered was 5(1-34). The median time to best response was 3 months (0.5-17) and 4 cycles (1-18). 17.5% of patients achieved CR, 14.6% PR, 13.6% HI with an overall response rate of 45.7% while 46.6% had SD and 7.8% PD. The median duration of response was 4 months (4-23). Improvement in WPSS was observed in 17.5%, no change in 32.5% and deterioration in 8.8%. The improvement in WPSS observed after treatment was statistically significant (p<0.0001), and with FAB, WHO and IPSS high risk categories. Significant was observed in 38% whereas in 26% of cases a stable response was observed. 28.3% and 27.3% of patients who were RBC and PLT transfusion dependently, became transfusion independent. Overall 26% of patients experienced transformation to AML within a median time of 9 months post treatment (1-90). 60.5% (69/114) patients remain alive, with an overall mortality rate of 37.7%. No significant association was observed between response to treatment and baseline clinical characteristics, prior therapy, transfusion dependency, and time to treatment onset since diagnosis. On the opposite patients who responded to treatment or had a stable disease had a significantly lower percentage of transformation to AML compared to patients with stable disease (p=0.002). Moreover transformation to AML positively correlated with lower neutrophil (p=0.004) and platelet counts (p=0.011), higher bone marrow blasts at baseline (p<0.0001), and with FAB, WHO and IPSS high risk categories. Conclusions. Azacitidine is a safe and effective treatment for intermediate and high risk MDS with an overall response rate of 45.7%. A significant improvement of WPSS score was observed following treatment while the risk of transformation to AML was significantly reduced in responding as well as patients with stable disease.

CLINICAL HETEROGENEITY AND OUTCOMES OF UNRELATED BONE MARROW TRANSPLANTATION IN 3 JAPANESE CHILDREN WITH FAMILIAL PLATELET DISORDER WITH PREDISPOSITION TO ACUTE MYELOGENOUS LEUKEMIA

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Background. Familial platelet disorder with predisposition to acute myelogenous leukemia (FPD/AML) is an autosomal dominant disorder characterized by thrombocytopenia and high risk for developing leukemia. Heterozygous germ-line mutations in RUNX1 have been identified in at least 52 FPD/AML families. As a contribution to the literature on this rare disorder, we present here the clinical courses and genetic studies in 2 Japanese families with FPD/AML. Case report: The proband of family I, who presented with isolated thrombocytopenia at the age of 1 year and was suspected of having moderate immune thrombocytopenic purpura (ITP), developed severe thrombocytopenia at the age of 7 years. Bone marrow findings showed features of myelodysplastic syndrome (MDS): presence of micromegakaryocytes and up to 14% of myeloblasts. Eight months later, he developed pancytopenia and was diagnosed with overt leukemia. Cytogenetic analysis showed normal karyotype and the immunophenotype of the blasts was CD7+, 13+, 33+ and 34+ and HLA-DR+. He received 3 courses of intensive chemotherapy. Because he did not achieve complete remission, he underwent unrelated donor marrow transplantation (UR-BMT), but relapsed 18 months later. At the age of 10 years, he underwent a second UR-BMT on relapse. The patient is still in remission 3 years later. The 3-year older sister of the proband also presented with moderate isolated thrombocytopenia at the age of 1 year and developed severe thrombocytopenia at the age of 11 years. Bone marrow findings showed features of ITP; increased numbers of immature megakaryocytes without blasts. Two years later, platelet number decreased below 2.0x10^4/μl and megakaryocytes disappeared in the bone marrow. This finding was quite different from the previous one. Cytogenetic analysis showed normal karyotype. Finally, she was diagnosed with MDS and received a successful UR-BMT at age 14 years. The patient is alive and well 2 years later. In contrast, their father has presented with only mild isolated thrombocytopenia (13.5x10^4/μl at age 36 years and 12.5x10^4/μl at 48 years). The proband of family II, who presented with moderate isolated thrombocytopenia at the age of 2 years, developed chronic myelomonocytic leukemia at age 9 years. She received a successful UR-BMT at age 12 years. The patient is alive and well 2 years later. The 2-year older sister of the proband also has a 10-year history of moderate isolated thrombocytopenia from the age of 1 year (8.5x10^4/μl at age 16 years) without bleeding tendency. Their father died of MDS at age 43 years. Sequencing analyses of RUNX1 revealed a heterozygous mutation in each family. Discussion: Clinical courses vary among FPD/AML patients within the same family. Our findings support the clinical heterogeneity of the disease as reported by other investigators. Because bone marrow findings in the early phase of FPD/AML mimic that in ITP, genetic analysis of RUNX1 is necessary to screen for FPD/AML. Finally, we consider that early indication of stem cell transplantation (SCT) leads to better outcome although the optimal conditioning regimen remains to be determined.
Constitutive Active Fibroblast Growth Factor Receptor 3 (FGFR3) with Lys650Glu Mutation Enhances the Bortezomib Sensitivity in Plasma Cell Malignancy

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Background. Previous cytogenetic studies in multiple myeloma (MM) patients have identified FGFR3 with K650E mutation as a frequent acquired abnormality. In contrast to the genomic alterations associated with the cytotoxic effect of bortezomib in malignant plasma cells, FGFR3 with K650E mutation enhances the bortezomib sensitivity in plasma cell malignancy.

Methods. Human plasma cell lines FR4 (MM) and RPMI8226 (MM) expressing FGFR3 wild type (WT), K650E or Y373C were treated with tunicamycin or cycloheximide.

Results. FGFR3 K650E transfected cells. The combination of bortezomib and ER stressor, tunicamycin, enhanced the cytotoxicity of bortezomib, resulting in the accumulation of BiP, Edem1 and CHOP in FR4 cells with FGFR3 K650E compared to FR4 cells with FGFR3 WT or Y373C. Similar results were observed by real-time PCR after treatment with bortezomib. As a result, BiP, Edem1 and CHOP were strongly induced by bortezomib in FR4 cells with mock, FGFR3 WT or FGFR3 Y373C. Similar results were obtained when RPMI8226 cells were used in place of FR4 cells.

Summary/Conclusions. This study indicated that FGFR3 with K650E mutation abrogated ER localization and enhanced the bortezomib-sensitivity in malignant plasma cells via ER stress pathways.

Early Relapse After Autologous Transplantation for Myeloma Is Characterized by Genes Mapping to Chromosome X

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Background. High dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) is currently the most widely accepted approach in newly diagnosed myeloma patients. HDT is known to be associated with a high incidence of relapse, which is a major cause of treatment failure. It is important to identify those who are likely to relapse early from ASCT, so that additional treatment could be given to modify the outcome. This study aims to examine the molecular basis of HDT resistance and to develop a predictive signature for patients at high risk of early relapse following ASCT. Methods. Gene expression profiling (GEP) data is available in 80 newly diagnosed myeloma patients who underwent front-line ASCT following induction therapy after informed consent. PFS was calculated from the date of HDT to the date of progression, with those dying without evidence of relapse censored at the time of death. OS was defined as the time from HDT to the date of death from any cause. Univariate Cox analyses were conducted on PFS to identify significant genes associated with early relapse with multiple testing adjustment. The independence of the genes from other prognostic factors was tested using multivariate Cox regression. Results. Nine genes were identified as being associated with early relapse after ASCT, among which five were on chromosome X. Three of the five chromosome X genes belonged to the cancer/testis gene family which has been shown to be prognostic in myeloma patients. NUDT11 has been reported to be involved in vesicle trafficking, DNA repair and apoptosis, and has been linked to drug resistance in some cancer types. The identified chromosome X genes were also significantly associated with shorter OS in our dataset.

Myeloma and other monoclonal gammopathies - Biology 1

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Background. Previous cytogenetic studies in multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) have identified FGFR3 with K650E mutation as a frequent acquired abnormality. In contrast to the genomic alterations associated with the cytotoxic effect of bortezomib in malignant plasma cells, FGFR3 with K650E mutation enhances the bortezomib sensitivity in plasma cell malignancy.

Methods. Human plasma cell lines FR4 (MM) and RPMI8226 (MM) expressing FGFR3 wild type (WT), K650E or Y373C were treated with tunicamycin or cycloheximide.

Results. FGFR3 K650E transfected cells. The combination of bortezomib and ER stressor, tunicamycin, enhanced the cytotoxicity of bortezomib, resulting in the accumulation of BiP, Edem1 and CHOP in FR4 cells with FGFR3 K650E compared to FR4 cells with FGFR3 WT or Y373C. Similar results were observed by real-time PCR after treatment with bortezomib. As a result, BiP, Edem1 and CHOP were strongly induced by bortezomib in FR4 cells with mock, FGFR3 WT or FGFR3 Y373C. Similar results were obtained when RPMI8226 cells were used in place of FR4 cells.

Summary/Conclusions. This study indicated that FGFR3 with K650E mutation abrogated ER localization and enhanced the bortezomib-sensitivity in malignant plasma cells via ER stress pathways.

Early Relapse After Autologous Transplantation for Myeloma Is Characterized by Genes Mapping to Chromosome X

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Background. High dose therapy (HDT) followed by autologous stem cell transplantation (ASCT) is currently the most widely accepted approach in newly diagnosed myeloma patients. HDT is known to be associated with a high incidence of relapse, which is a major cause of treatment failure. It is important to identify those who are likely to relapse early from ASCT, so that additional treatment could be given to modify the outcome. This study aims to examine the molecular basis of HDT resistance and to develop a predictive signature for patients at high risk of early relapse following ASCT. Methods. Gene expression profiling (GEP) data is available in 80 newly diagnosed myeloma patients who underwent front-line ASCT following induction therapy after informed consent. PFS was calculated from the date of HDT to the date of progression, with those dying without evidence of relapse censored at the time of death. OS was defined as the time from HDT to the date of death from any cause. Univariate Cox analyses were conducted on PFS to identify significant genes associated with early relapse with multiple testing adjustment. The independence of the genes from other prognostic factors was tested using multivariate Cox regression. Results. Nine genes were identified as being associated with early relapse after ASCT, among which five were on chromosome X. Three of the five chromosome X genes belonged to the cancer/testis gene family which has been shown to be prognostic in myeloma patients. NUDT11 has been reported to be involved in vesicle trafficking, DNA repair and apoptosis, and has been linked to drug resistance in some cancer types. The identified chromosome X genes were also significantly associated with shorter OS in our dataset.
which is independent from any other known prognostic factors such as β2m, αβ, del(13), gain(1q) and adverse chr14 translocations identified by FISH (p < 0.05). The prognostic value of the genes identified was also validated in an external cohort of patients treated with ASCT. Conclusion. By analyzing GEP data we identified genes significantly associated with high risk of early relapse following ASCT, most of which mapped to chromosome X. Upon further investigation, these genes could give insight into the biology underlying HDT resistance. The development of a predictive signature based on the identified genes is currently under-going and will be presented at the conference.

0277

This abstract has been withdrawn.

0278

CANONICAL AND NON CANONICAL HEDGEHOG PATHWAY IN THE PATHOGENESIS OF MULTIPLE MYELOMA

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Background. The Hedgehog (Hh)-pathway is required for cell fate-determination during the embryos life, cell growth and differentiation in the adult organism, and is conserved in several tissues including the adult organism. In mature tissues it regulates tissue homeostasis and repair and, in those tissues undergoing constant renewal as skin, colon, liver and blood it is also implicated in maintaining a stem/progenitor cell compartment. In this context, it is easy to understand how Hh-pathway deregulation may cause development defects during the embryonic life, while leads to tumorigenesis during the adult life for a stem cell pool expansion or for onset, within more differentiated cells, of mutations affecting the normal growth-regulatory mechanisms. We already showed that an aspect of plasma cells (PCs) malignant transformation is the aberrant expression of developmental genes as those of Wnt- and Hh-pathways. Several evidences support a role of Hh-signaling in regulating a stem cell niche also in Multiple Myeloma (MM). In contrast, the application of the same technology in attempt to predict clinical outcome has been less successful with the identification of heterogeneous molecular signatures. Aim. This approach was aimed at the identification of robust and reproducible signatures associated with prognosis across independent datasets. Methods. We have reconstructed gene regulatory networks in a panel of 1883 samples from MM patients profiled on Affymetrix platform, derived from seven publicly available gene expression sets. The transcriptional networks were reconstructed using ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks). Critical analysis of network components was applied to identify genes playing an essential role in transcriptional networks, which are conserved between datasets, and proportional hazard models were used to evaluate the association of each gene with outcome. The correlation with overall survival was tested in three of the seven datasets for which clinical data were available. Results. The network critical analysis revealed that i) CCND4 and CCND2 were the most critical genes; ii) among the top critical genes CCND2, AIF1 and BLNK had the largest number of connections shared among the datasets; and iii) robust gene signatures with prognostic power were derived from the most critical transcripts and from shared primary neighbors of the most connected nodes. In particular a “critical-gene”model, comprising FAM53B, KIF12B, WHSC1 and TEMPO, and a “neighbor-gene”model, comprising BLNK shared neighbors CSGALNACT1 and SLC7A7, predicted survival in all datasets with follow-up information. Conclusion. The reconstruction of gene regulatory networks in a large panel of primary tumors suggested novel molecular mechanisms central to MM biology and identified specific genes with prognostic importance.

0279

THE ANALYSIS OF TRANSCRIPTIONAL NETWORK IN MULTIPLE MYELOMA REVEALS CRITICAL GENES WITH BIOLOGICAL AND CLINICAL IMPLICATIONS

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Background. The combined use of microarray technologies and bioinformatics analysis has improved our understanding of biological complexity of multiple myeloma (MM). In contrast, the application of the same technology in attempt to predict clinical outcome has been less successful with the identification of heterogeneous molecular signatures. Aim. This approach was aimed at the identification of robust and reproducible signatures associated with prognosis across independent datasets. Methods. We have reconstructed gene regulatory networks in a panel of 1883 samples from MM patients profiled on Affymetrix platform, derived from seven publicly available gene expression sets. The transcriptional networks were reconstructed using ARACNe (Algorithm for the Reconstruction of Accurate Cellular Networks). Critical analysis of network components was applied to identify genes playing an essential role in transcriptional networks, which are conserved between datasets, and proportional hazard models were used to evaluate the association of each gene with outcome. The correlation with overall survival was tested in three of the seven datasets for which clinical data were available. Results. The network critical analysis revealed that i) CCND4 and CCND2 were the most critical genes; ii) among the top critical genes CCND2, AIF1 and BLNK had the largest number of connections shared among the datasets; and iii) robust gene signatures with prognostic power were derived from the most critical transcripts and from shared primary neighbors of the most connected nodes. In particular a “critical-gene”model, comprising FAM53B, KIF12B, WHSC1 and TEMPO, and a “neighbor-gene”model, comprising BLNK shared neighbors CSGALNACT1 and SLC7A7, predicted survival in all datasets with follow-up information. Conclusion. The reconstruction of gene regulatory networks in a large panel of primary tumors suggested novel molecular mechanisms central to MM biology and identified specific genes with prognostic importance.

0280

INHIBITION OF PROTEIN KINASE CK2 AFFECTS THE HOMEOSTASIS OF THE UNFOLDED PROTEIN RESPONSE PATHWAYS IN MULTIPLE MYELOMA CELLS AND EMPOWERS THE CYTOTOXIC EFFECT OF HSP90 INHIBITORS

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Background. Hsp90, a central chaperone molecule involved in the maturation and folding of several cellular client proteins, is essential for malignant plasma cell survival. Hsp90 inactivation in multiple myeloma (MM) cells has been shown to cause perturbation of the ER stress/unfolded protein response (UPR), eventually triggering the apoptotic cascades. Protein kinase CK2 is an important regulator of Hsp90 activity phosphorylating the Hsp90 cochaperone Cdc37 stabilizing a macromolecular complex containing Hsp90, Cdc37 and client proteins. We previously described that CK2 is over-expressed in a fraction of MM patients and is an essential MM pro-survival molecule. Currently, phase I clinical trials are ongoing with Hsp90 inhibitors and orally available ATP-competitive CK2 specific inhibitors. Aim. We have here investigated the role of CK2 in the ER stress/UPR pathways and in Hsp90 inhibition.
inhibition-induced apoptosis in MM cells. We analyzed CK2 activity upon ER stress and the consequences of the effects of CK2 inhibition/silencing on ER stress induced-apoptosis triggered by chemicals and Hsp90 inhibitors. Methods. MM cell lines, human bone marrow stromal cells and plasma cells from patients were exposed to geldanamycin or 17-AAG (17-(demethoxy)-17-allylamo geldanamycin) (Hsp90 inhibitors) and CK2 inhibitors K27 and CX4945. RNA interference was used to silence the CK2 catalytic α subunit. Thapsigargin and Tunicamycin were used to trigger ER stress. Annexin V and propidium iodide staining and analysis of FAPR cleavage were employed to assess cell growth and viability. UPR related signaling pathways were studied with western blot and real-time-polymerase chain reaction analysis. Results. Down-regulation of the catalytic CK2α subunit with chemical inhibitors or RNA interference resulted in modifications of the main UPR regulating signaling cascades: a reduction of IRE1α protein levels; a reduction of BiP/GRP78 chaperone protein levels; an increase of PERK activity and phospho eIF2α levels. CK2 partly localized to the ER and the ER-stressor Thapsigargin triggered its kinase activity. CK2 inactivation enhanced Thapsigargin-induced apoptosis and opposed CHOP/GADD153 and IRE1α rise. Treatment of CK2-inhibited/silenced MM cells geldanamycin or 17-AAG resulted in a much more pronounced reduction of IRE1α protein levels; a marked inhibition of GA or 17-AAG-triggered BiP/GRP78 protein level raise; a more evident increase of eIF2α phosphorylation. Of note, CK2 plus Hsp90 inhibition was followed by apoptotic cell death to a much greater extent than that obtained with the single inhibition of the two molecules. Noteworthy, these effects were also reproduced upon modeling the MM bone marrow microenvironment by co-culturing MM cells with BM stromal cells and on plasma cells isolated from MM patients. Results. Initially, we demonstrated that CK2 inhibition leads to a reduction of IRE1α/HSP90/CDC37 complexes in MM cells, a phenomenon that could justify the reduced IRE1α half-life in MM cells. Summary/Conclusions. These data highlight the importance of CK2 in tuning HSP90 function and the ER stress/UPR cascades in MM cells. In view of the very recent development of phase I clinical trials with both CK2 inhibitors and Hsp90 inhibitors as anti-MM agents, our results might provide useful insights to better set the groundwork in designing novel combination treatments for this disease.

**0282**

**AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA: IMPACT OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) IN GENES INVOLVED IN INFLAMASSOME AND MiRNA NETWORK IN SURVIVAL AND PROGRESSION**

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Background. Polymorphisms (SNPs) in proteins involved in cytosolic macrocomplex with regulatory functions in the immune system have previously shown to have prognostic impact after stem cell transplantation. Otherwise, SNPs in miRNA proteins pathway and in the target genes binding sites (miR-SNPs) have been observed with different prognostic implications in some tumors. However, data in multiple myeloma (MM) has ever been reported. Patients and Methods. One hundred and thirty seven patients with chemosensitive MM (76M/64F, median age 55 years) intensified with autologous stem cell transplantation (ASCT) have been studied in one institution. The patients achieved at least a minimal response after one (117) or two (20) induction regimens prior to ASCT. The genes (SNPs) evaluated in genomic DNA by allelic discrimination (TaqMan assays) were NLRP2 (rs1045684), NLRF3 (rs10925027), ATBP1 (rs3925927) and EB800 (rs20551) for innate immune system, and KRT18 (rs3660), AFF1 (rs17702651), EAM179b (rs1053667) and XPO5 (rs11077) for miR-SNPs. Results. Overall survival (OS) was significantly longer in patients with SNPs in KRT18 (rs3660; p=0.029), NLRP2 (rs1045684; p=0.053) and XPO5 (rs11077; p=0.012)(Figure). A correlation of this latter polymorphism in XPO5 with progression-free survival (PFS) was also observed (p=0.015). This miR-SNP retained its prognostic impact on PFS and OS when a Cox
0283 THE EFFECT OF LENALIDOMIDE AND DEXAMETHASONE COMBINATION ON BONE REMODELING OF RELAPSED/REFRACTORY MYELOMA: FINAL RESULTS OF TWO STUDIES OF THE GREEK MYELOMA STUDY GROUP WITH 205 PATIENTS

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Background. Lenalidomide plus dexamethasone (RD) is very effective for the management of refractory/refractory multiple myeloma (MM). However, there is limited information for the effect of RD on bone remodeling of MM patients. Aims. We performed both a retrospective analysis and a prospective study to evaluate the effect of RD regimen on bone remodeling in relapsed/refractory MM. Methods. Firstly, we evaluated 106 consecutive patients (54M/52F, median age 68 years) who received lenalidomide at the standard dose of 25mg PO daily (or adjusted to creatinine clearance) on days 1-21 of a 28-day cycle in combination with dexamethasone at a dose of 40mg PO on days 1-4 and 15-18 for the first four cycles and only on days 1-4 thereafter. All patients were under zoledronic acid both pre- and during treatment period. The following serum indices of bone metabolism were measured on day 1/cycle 1, and then on day 28 of cycles 3 and 6: (i) osteocalcin and β-carboxypropeptide of collagen type I C-propeptide (OC); (ii) bone-specific alkaline phosphatase (ALP); (iii) C-terminal telopeptide of type I collagen (CTX); (iv) bone formation markers: bone-specific alkaline phosphatase (bALP) and osteocalcin (OC). These markers were also evaluated in the prospective study. Results. The general pro-survival decrease in osteocalcin and bone-specific alkaline phosphatase levels in MM cells. Remarkably, the siRNA-mediated knockdown significantly reduced MM cell proliferation, as shown by cell cycle analysis with propidium iodide staining. We observed that unwanted side effects of BZ, such as delayed diagnostic; and by allowing a closer monitoring of the applied chemotherapy.

1 expression by lenalidomide. On the contrary, VRD enhances bone formation, at least partially due to a significant reduction of Dkk-1, reflecting the strong anabolic effect of bortezomib in MM patients.

0284 INHIBITION OF PROTEIN KINASE CK2 ENHANCES THE CYTOTOXIC EFFECTS OF BORTEZOMIB ON MULTIPLE MYELOMA CELLS

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Background. Multiple Myeloma (MM) cells are exquisitely sensitive to the cytotoxic effects of proteasome inhibitors (PI). Bortezomib (BZ) is a first-in-class PI currently widely used in the therapy of MM patients. BZ causes MM cell apoptosis through different mechanisms which are only partially known. The pro-survival protein kinase CK2 has been implicated in human cancer and phase I clinical trials are ongoing utilizing oral ATP-competitive CK2 inhibitors in MM and other tumors. Aims. In this study we have investigated the role of CK2 in the regulation of BZ-induced MM cell death. We aimed to assess apoptosis and proliferation of MM cells exposed to BZ and CK2 inhibitors. We investigated pro-survival signaling pathways associated with MM cell resistance to BZ and chemotherapeutic agents. Method. MM cell lines, human bone marrow stromal cells and freshly isolated plasma cells from patients were cultured and exposed to BZ and the CK2 inhibitors K27 and CX4945. Cell growth and viability was assessed upon the different treatments by annexin V and propidium iodide staining. MTI-assays, evaluation of mitochondrial membrane potential, apoptosis assays and cytokine production were also performed. Results. BZ-induced apoptosis and cell cycle arrest were significantly inhibited by simultaneous inhibition of CK2. This effect was observed both in MM cell lines grown in suspension, in a model of bone marrow microenvironment as well as in malignant plasma cells isolated from MM patients. Mitochondrial membrane potential measurements revealed that CK2 inhibition enhanced BZ-triggered intrinsic apoptotic cell death. Also, CK2 inhibition together with BZ treatment was associated with reduced MM cell proliferation, as shown by cell cycle analysis with propidium iodide staining. We observed that unwanted side effects of BZ treatment were the activation of the MM growth-promoting NF-κB and STAT3 cascades. Survival signaling pathways were studied with WB analysis and RT-PCR. Results. BZ-induced apoptosis and cell cycle arrest were significantly inhibited by simultaneous inhibition of CK2. This effect was observed both in MM cell lines grown in suspension, in a model of bone marrow microenvironment as well as in malignant plasma cells isolated from MM patients. Mitochondrial membrane potential measurements revealed that CK2 inhibition enhanced BZ-triggered intrinsic apoptotic cell death. Also, CK2 inhibition together with BZ treatment was associated with reduced MM cell proliferation, as shown by cell cycle analysis with propidium iodide staining. We observed that unwanted side effects of BZ treatment were the activation of the MM growth-promoting NF-κB and STAT3 signaling pathways. The rise in the levels of the unfolded protein response-associated kinase Ire1α. These changes could lend MM cells the ability to escape the cytotoxic effects of BZ. Oppositely, CK2 inhibition was associated with a strong reduction of phospho-p65 NF-κB, phospho-STAT3 and Ire1α levels in MM cells. Remarkably, the simultaneous treatment of BZ with CK2 inhibitors was accompanied with a significant reduction of BZ-triggered p65 NF-κB and STAT3 activation and we found that CK2 inhibition was also able to hamper the BZ-induced rise in Ire1α levels. Summary/Conclusions. These results indicate that protein kinase CK2 can antagonize BZ-induced apoptosis pathways critical signaling pathways in MM cells, such as the NF-κB and STAT3 cascades. Our findings indicate that CK2 inhibition could represent a rational therapeutic strategy to be tested in designing novel BZ-based anti-MM combination therapies.

0285 USE OF SPECIFIC IMMUNOGLOBULIN HEAVY/LIGHT CHAINS PAIRS FOR THE DIAGNOSTIC AND FOLLOW-UP OF MULTIPLE MYELOMA PATIENTS: NORMAL RANGES AND ASSAY UTILIZATION

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Background. Multiple myeloma (MM) is a B cell disorder and is characterized in most of the cases by the production of a monoclonal protein (MP). The detection and quantification of the MP by serum protein electrophoresis is the most used technique for the screening of monoclonal gammopathies. However, this can often be difficult, especially in cases where the paraprotein is of low amount and in cases where the band is masked by other proteins. Immunofixation (IFE) improves sensitivity to the detection protocol but it is not a quantitative method. Therefore, not suitable for the follow-up. More accurate methods could benefit the patient in several ways: by avoiding problems related to a delayed diagnostic; and by allowing a closer monitoring of the applied therapy, and by doing so helping in treatment decisions such as when
ANKHD1 IS HIGHLY EXPRESSED IN MULTIPLE MYELOMA AND PLAYS A ROLE IN PROLIFERATION AND IN THE ACCUMULATION OF CELLS IN THE S PHASE OF CELL CYCLE

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Background. Ankyrin-repeat-containing proteins regulate multiple cellular functions including transcription, cell cycle and cell survival. Ankyrin repeat and KH domain-containing protein 1 (ANKHD1) is one such protein ubiquitously expressed in normal human tissues and has a varied and high expression in cancer, including acute leukemias. Multiple myeloma (MM) is a plasma cell malignancy preliminary localized in the bone marrow and characterized by its capacity to disseminate. Previous study by our group showed that ANKHD1 is highly expressed in plasma cells of MM patients compared with normal individuals; however its role in the development of MM is still undetermined.

Aim. The present study was aimed to study the expression of ANKHD1 mRNA and protein in a panel of multiple myeloma cell lines. In addition we used lentiviral mediated RNA interference technique to down regulate the expression of ANKHD1 gene in human myeloma cell line U266 and studied its effect on apoptosis, proliferation and cell cycle.

Methods. MM cell lines U266, MM1S, MM1R and RPMI 8266 were used. ANKHD1 mRNA and protein expression were evaluated using real-time RT-PCR and Western blot. Localization of ANKHD1 in cells were analysed by laser confocal microscopy. Specific shRNA-expressing lentiviral vector to ANKHD1 or LacZ gene (control) was designed and used for transduction in U266 cell line. Quantitative PCR (qPCR) and Western blot analysis were performed to determine the inhibition of ANKHD1 expression 48 hours post transduction. Proliferation was analyzed by MTT assays, apoptosis by Annexin-V and propidium iodide (PI), cell cycle by incubation with PI and RNase A buffer and flow cytometry. Results. ANKHD1 mRNA and protein was found to be highly expressed in all MM cell lines as evident by q-PCR and Western blot when compared to K652, a leukemia cell line (positive control). Confocal microscopy showed ANKHD1 to be predominantly localized in cytoplasm of MM cell lines. Lentiviral mediated ANKHD1 shRNA downregulated ANKHD1 mRNA expression and protein level significantly, with a downregulation of 88% and 92%, respectively when compared with control cells (P<0.0001). MTT assays showed that the proliferation of MM cells was significantly reduced by 70% in ANKHD1 knockdown cells when compared with control cells (P<0.0001). Cell cycle analysis showed an increase of cells in S phase (59.3±1.5 versus 49.4±2.4; P<0.0001) in ANKHD1 knockdown cells as compared to control cells. However, annexin-V analysis showed no significant increase in apoptosis of cells on silencing ANKHD1 expression. Conclusion. ANKHD1 is highly expressed in MM cell lines studied and is predominantly localized in the cytoplasm. Downregulation of ANKHD1 in MM cell line U266, effectively caused decrease in cell proliferation and increase in S phase cells suggesting that ANKHD1 plays a role in cell proliferation and in the accumulation of S phase cells. Further studies are being carried out to elucidate the underlying mechanisms.

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99mTc-TRICARBONYL-TOCILIZUMAB: A NEW MOLECULAR IMAGING AGENT IN MULTIPLE MYELOMA

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Background. Interleukin-6 (IL-6) is a key molecule in the pathogenesis of multiple myeloma (MM), resulting in myeloma cell proliferation. Conjugation of IL-6 receptor (IL6R) to MM cells has emerged as a molecular target for imaging as well as for treatment. Tocilizumab, a humanized anti IL6R monoclonal antibody has been conjugated with 99mTc through HYNIC showing good stability in vitro and in vivo. Conjugation of 99mTc-Tocilizumab by means of tricarbonyl could be a suitable option as imaging agent in MM, with the advantage of not requiring derivatization previous to its labeling. Aims. Development of 99mTc (CO) 3 -Tocilizumab. Evaluation as imaging agent in MM. Methods. 1ml of 99mTc-pertechnetate solution was added to the IsoLink carbonyl labeling agent (Mallinckrodt). 1 mg Tocilizumab (Roche) was incubated with 1ml of 99mTc (CO) 3 -OH) 2 at pH 7, during 45 min at 57°C. Radiochemical purity was controlled by chromatographic systems: ITLC/NaCl 0.9%, Whatman 5MM/MEK, ITLC in BSA/EtOH-NH3-H2O (2:1:5) as stationary and mobile phase and by HPLC using size exclusion column and a phosphate buffer 0.01 M, pH7 isocratic gradient as mobile phase at 1ml/min. Stability of 99mTc (CO) 3 -Tocilizumab was evaluated in PBS and challenged with hydrogen peroxide during 24 h. Binding studies to U266 MM cells were performed incubating 200000 cpm of the conjugate with 1000000 cells in 1ml culture medium at 30, 60 and 120 min. Specificity of binding was supported by competition experiments using unlabeled antibody. Tocilizumab was derivatized with FITC and purified by PD10 column. Laser scanning confocal microscopy was done with an excitation/emission wavelength of 488/530 nm. Fluorescent images were obtained. Atomic force microscopy imaging of U266 cells were done. Biodistribution studies were performed at 24 h in CD1 normal mice (n=5). Each mice was in-
jected with 37 MBq of $^{99m}$Tc(CO)$_3$-Tocilizumab and sacrificed 24 h after injection. Organs of interest were collected. Results: Radiochemical purity was 91.0 ± 1.1% and 93.5 ± 0.5% at 1 and 2 h respectively, remaining stable at 24 h, showing no significant transchelation. Competition experiments using cold antibody showed a reduction in binding to U266 cells superior to 50%, confirming the specificity of binding. Confocal microscopy provided the ability to recognize the IL6R in U266 myeloma cells. A reduction in binding to U266 cells was (4.2) %act/g, liver (3.4) %act/g, kidney (1.2) %act/g and spleen (1.1) %act/g uptake with hepatic and renal elimination. Conclusion: Tocilizumab was easily labeled to 99mTc through tricarbonyl with good stability, radiochemical purity and specificity. These results were similar to those obtained in its conjugation through HYMIC (2). $^{99m}$Tc(CO)$_3$-Tocilizumab may be a useful imaging agent in MM.

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0288 EXTRACELLULAR MATRIX REMODELING AND STROMAL CELL-DERIVED TUMOR PROMOTION IN THE BONE MARROW REFLECT THE PROGRESSION OF MGUS TO MULTIPLE MYELOMA

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Background. The pathogenesis of multiple myeloma (MM) is regarded as a multistep process, in which an asymptomatic stage of MGUS precedes virtually all cases of MM. Molecular events leading to transition from MGUS to MM are still poorly defined. Genetically, MGUS plasma cells resemble MM plasma cells in many features, and the clinically apparent step-wise progression of MGUS to MM is poorly reflected by genetic aberrations. Aim. We hypothesized that the bone marrow microenvironment is critically involved in the pathogenesis of monoclonal gammopathies. Therefore, we performed a comparative proteome profiling study and investigated the contribution of bone marrow fibroblast precursors to cell disease progression in MM. Methods. Primary bone marrow fibroblasts from patients with MGUS and MM were compared to control fibroblasts obtained from hip replacement surgery. Primary cells were cultured for 3 to 5 passages, characterized by immunophenotyping (FACS analysis), fractionated into cytoplasmic, nuclear and secreted protein fractions and then analyzed using shotgun proteomics. Confirmatory experiments were performed using Western blotting. Results. Strikingly, a group of extracellular matrix (ECM) proteins, ECM receptors and ECM-modulating enzymes was found to be progressively up-regulated from controls to MGUS and to MM. These proteins include laminin 8, lysyl hydroxylase 2, integrin alpha-5, macrophage mannose receptor 2, PAI-1 and MMP-2. Additionally, the growth factors peristin and stem cell growth factor as well as PDGF-receptor beta showed a similar progression-related pattern. Conclusion. Our results indicate that ECM remodeling and stromal cell-derived tumor promotion in the bone marrow takes place already at the level of MGUS and becomes even more pronounced in MM. Thus, for the first time, marker proteins could be identified indicating a step-wise progression from MGUS to MM.

0289 TUMOR-PRIMED NATURAL KILLER CELLS FROM PATIENTS WITH MULTIPLE MYELOMA LYSE AUTOLOGOUS, NK-RESISTANT, BONE MARROW-DERIVED MALIGNANT PLASMA CELLS

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Background. Natural killer (NK) cells are cytotoxic lymphocytes able to kill tumor cells and virus-infected cells. Human resting NK cells can be activated by co-culture with NK-resistant CTV-1 cells. These tumor-activated cells (TaNK) are cytotoxic to a range of NK-resistant tumor cells in vitro. This potential, has not been explored in multiple myeloma (MM). Aim. The current study was designed to assess the relative function in vitro of NK and TaNK cells from MM patients compared to normal controls in the lysis of tumor cell-lines and freshly isolated autologous NK cells. Methods. Freshly isolated CD56+ NK cells from normal donors and 18 MM patients, separated with CD56 immunomagnetic Microbeads were co-incubated with CTV-1 cells or lysates there from, overnight at 37°C, 5% CO2, to generate TaNK cells. For the cytotoxicity assay, the erythroleukemia cell-line K562 with known sensitivity to NK lysis was used as a positive control, the Burkitt’s lymphoma cell-line Raji, known to be refractory to NK lysis was used as a negative control of NK killing. The Myeloma cell-line U266 as well as freshly isolated bone marrow (BM) autologous and allogeneic CD138+ plasma cells from MM patients were used to evaluate the sensitivity of plasma cells to MM patients’ NK and TaNK lysis. Cytotoxicity was measured in a 4-h assay. Loss of membrane integrity was measured by ingestion of To-Pro-3 iodide as determined by flow cytometry. Bone marrow plasma cells, were acquired after electronic gating on the CD138+ cells, and the mononuclear fraction of CD138 positive/To-Pro-3 iodide positive cells from the samples was determined. Background. Target-cell death was determined from cells incubated in the absence of effector cells and the “percent lysis - CD138+” was calculated by subtraction of the background cell death. To determine the specificity of malignant plasma cell lysis, autologous cell lines were used in the same analysis. In addition, the “percent lysis - control cells” determined as above. This was subtracted from the “percent lysis - CD138+” to give a “percent specific lysis”. Results. We have demonstrated that TaNK cells from MM patients lyse several myeloma targets, including autologous and allogeneic CD138+ myeloma cells whilst sparing CD138-ve BM cells. Myeloma patients’ TaNK-induced lysis of the U266 cell-line was significantly higher compared to normal controls (median specific lysis 79.1% vs 69.5%) (p=0.003). In addition, TaNKs induced substantial lysis of autologous and allogeneic CD138+ myeloma cells (median specific lysis 52.5% and 57.4%, respectively). The percentage of specific lysis did not correlate with the most important disease characteristics (age, gender, risk high-risk cytogenetics), nor with the disease status and anti-myeloma treatment, including novel agents and dexamethasone. Summary/Conclusions. Tumor-primed NK cells are able to induce substantial lysis of myeloma targets including autologous and allogeneic CD138+ myeloma plasma cells and could be an additional therapeutic approach in MM, particularly in the era of novel agents.
Table 1.

| Mean mRNA levels of telomere-associated genes in patients with respect to normal controls were observed (p<0.04). The comparison between both entities showed higher POT1 (p=0.0007), RAP1 (p=0.02), TIN2 (p=0.008) mRNA levels in MM with respect to MGUS patients. In both pathologies an up-regulation of hTERT and a positive association with RAP1 and TFP1 (p<0.0002) were found. For a better analysis, patients were divided into two groups according to hTERT levels using ROC curves: hTERT mRNA levels <1.08 (Group A, GA) and >1.08 (Group B; GB). Table 1 shows the global analysis of all shelterin genes, hTERT and TL. In both entities, an increased gene expression in GB with respect to GA, with significant differences in MM for TFP1 and TRF2 as well as shorter TL (p<0.01) was observed. TFP1 also showed significant differences between GBs from MM and MGUS (p=0.008). Higher levels of RAP1 (p=0.009), POT1 (p=0.002), and TIN2 (p=0.01) in GAs from MM and MGUS were also found. In MM, the analysis of clinical characteristics showed a negative association between hemoglobin and POT1 and RAP1 expression (p<0.03), while the percentage of bone marrow infiltration was positively correlated with POT1 and TFP1 mRNA levels (p<0.03). RAP1 expression was also positively associated with calcium and creatinine levels (p<0.001). Although non-significant, a shorter overall survival in MM patients of GB compared to GA was observed. Conclusion. Our findings show a global modification in the expression of telomere-associated genes in MM and MGUS, suggesting that the up-regulation of most of the shelterin components may contribute to the stabilisation of short telomeres by delaying/repressing the telomere damage signals, contributing to the development and/or progression of the disease.

Background. Monoclonal gammopathies (MG) are characterised by presence of different numbers of clonal plasma cells (PC), lower in monoclonal gammopathy of undetermined significance (MGUS) and higher in multiple myeloma (MM). Discrimination between normal polyclonal PC (N-PC) and abnormal clonal PC (A-PC) is important in time of diagnosis and especially after treatment (transplantation etc.). Expression of surface markers CD19 and CD56 on PCs is widely used for this discrimination, but unfortunately in some cases is not enough sufficient. Aims. Verification and validation of 8-color flow cytometry protocol for clonality assessment of PC and B cell subsets in MGs. Methods. Analyses of 22 MG patients in various stages of diagnosis and treatment were done (14 newly diagnosed MGUS/MM, 6 treated MM and 2 relapsed/progressed MM patients). Combination of CD38/CD138/CD19/CD56/CD45/CD27/ckappa/clambda was used to detect clonality of PCs subpopulations, mature B cells and memory B cells by flow cytometry. Results. There was found 0.4% (0.04-13.40) [median (min-max)] of CD38+CD138+ PCs in bone marrow. Only N-PCs (CD19 CD56 PCs) were found in 4 patients (2 new diagnoses, 1 progression and 1 post-treatment) and only clonal A-PCs (CD138+ CD56+ PCs) were found in 5 patients (1 new diagnosis, 1 progression and 1 post-treatment). Remaining cases were characterized by mixture of N-PCs and A-PCs, but detailed analysis of PC subpopulations using cytoplasmic kappa/lambda expression shown that not all CD19 PCs and/or CD56 PCs are clonal as was expected. This detailed analysis could be very useful in minimal residual disease (MRD) monitoring. Simultaneous analysis showed presence of clonal mature B cells and clonal memory B cells as well in 5 cases (4 new diagnoses and 1 post-treatment), so presence of lymphoproliferation should be verified in these patients. Summary/Conclusions. Analysis using combination of surface and intracellular markers in 8-color setting can improve detection even small clone of pathological PCs and together with B cell assessment can be very important for determination of diagnosis and monitoring after treatment. Supported by GACR 301/09/P457, MSMT LC06327, MSM021622434, IGA 10408-3, IGA 10406-3 and GACR P304/10/1395 grants.

0291

CLONALITY ASSESSMENT USING 8-COLOR FLOW CYTOMETRY

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Background. Multiple myeloma (MM) is a lymphoproliferative disease characterized by clonal expansion of neoplastic plasma cells within the bone marrow. The genome of malignant plasma cells is extremely unstable characterized by a complex combination of structural and numerical abnormalities. It is suggested that centrosome abnormalities, of which centrosome amplification is the most prominent, occur early in MM pathogenesis and increase with disease progression. Centrosome amplification is therefore associated with deregulation of cell cycle, mitosis, DNA repair and proliferation. Aims. The objective of our study was to evaluate changes in expression profile of genes involved in centrosome structure/function in MM with known role in oncogenesis. Methods. 57 patients were evaluated by gene expression profiling of PCs. CD188+ cells were separated by MACS. Total RNA was transcribed into cDNA (Ambion WT Sense Target assay), labeled and hybridized to the Affymetrix GeneChip Human Gene ST 1.0 array according to a manufacture protocol. Acquisition of Affymetrix array images, RMA normalization algorithm and hierarchical clustering algo-
B cells in the PB of MM patients. was applied to determine the number of CT antigen-specific memory and PB samples from 97 healthy donors were screened for antibody re-

further showed that antibody responses were restricted to regions of

C2/CT10-specific immune responses in the patients were found to be
detectable in 33 (15%) of MM patients. In agreement with this

detectable humoral responses were directed against MAGE-C2/CT10

progression of MM.

Acknowledgments. This study was supported by grants IGA NS10207, IGAT1154 and MSM0021622434.

0293

CANCER-TESTIS ANTIGENS OF THE MAGE FAMILY INDUCE SPONTANEOUS HUMORAL RESPONSES IN PATIENTS WITH MULTIPLE MYELOMA

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Background. We have previously shown that cancer-testis (CT) anti-
gens are specifically expressed in the bone marrow (BM)-infiltrating plasma cells of patients with multiple myeloma (MM) and that certain members of the MAGE family are the CT antigens most commonly de-
tected in MM. In addition MAGE genes, such as MAGE-C1/C17, MAGE-A1/MAGE-B10 and MAGE-A5, seem to independently promote the progression of MM. Aims. In this study, we investigated for the first time the occurrence of spontaneous humoral responses against these promising targets for the antigen-specific therapy of MM. Methods. Per-
nipheral blood (PB; N=1547) plasma samples from 225 MM patients and PB samples from 97 healthy donors were screened for antibody re-

sponses against MAGE-A1, MAGE-A5, MAGE-A8, MAGE-A11, and MAGE-C2/CT10 by ELISA and western blot. A B cell ELISPOT assay was applied to determine the number of CT antigen-specific memory B cells in the PB of MM patients. Results. MAGE-A11-, MAGE-A1-, MAGE-A5-, and MAGE-A3-specific antibody responses occurred in 17 (7.6%), 5 (2.2%), 4 (1.7%), and 3 (1.3%) MM patients respectively, at least once throughout the course of their disease. The most commonly detectable humoral responses were directed against MAGE-C2/CT10 being present in 33 (15%) of MM patients. In agreement with this finding, we were also able to demonstrate for the first time the presen-
ce of MAGE-C2/CT10-specific memory B cells in the PB of MM pa-
tients by ELISPOT. In a western blot analysis, spontaneous MAGE-

C2/CT10-specific immune responses in the patients were found to be highly specific for both natural and recombinant protein. Epitope map-
ping in an ELISA using overlapping MAGEC2/CT10 20mer peptides further showed that antibody responses were restricted to regions of the full-length protein spanning amino acids 40-60, 160-180, 180-200, and 270-290. MAGEC2/CT10-specific antibodies consisted mainly of IgG2 and to a lesser extent of IgG1, IgG3 and IgG4 subtypes. Conclu-
sions. Cancer-testis antigens of the MAGE family, especially MAGE-

C2/CT10, are capable of inducing spontaneous humoral response in MM patients. These antigens represent promising targets for the antigen-specific immunotherapy of MM but might also be of use as di-
agnostic and/or prognostic parameters for myeloma represent potential targets for immunotherapy in patients with multiple myeloma, vacci-
nation targeting this kind of antigen could remarkably enhance the cor-
responding response.

Red blood cells - Clinical and transfusion

0294

A MULTICENTER, OPEN LABEL STUDY OF LENALIDOMIDE AND PREDNISONE (RP) FOLLOWED BY LENALIDOMIDE, MELPHALAN AND PREDNISONE (MPR) IN NEWLY DIAGNOSED ELDERLY MULTIPLE MYELOMA PATIENTS

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Background. The combination of Melphalan-Prednisone-Lenalido-

mide (MPR) has shown promising results in elderly newly diagnosed myeloma patients. In young myeloma patients, low-dose chemotherapy (induction) precedes high-dose chemotherapy (autologous transplanta-
tion consolidation). This approach reduces tumor mass, with few side effects, before achieving the maximum cyto-reduction with transplanta-
tion consolidation. The same approach has been designed for the eld-

yry patients: lenalidomide plus corticosteroids precedes consolidation with MPR. Aims. To evaluate the safety and efficacy of Lenalidomide-

Prednisone (RP) as induction, followed by Melphalan-Prednisone-

Lenalidomide (MPR) as consolidation and RP as maintenance in elderly myeloma patients. Methods. Unfit patients with newly diagnosed symptomatic myeloma older than 65 years were enrolled in a two-

stage phase II clinical trial designed according to Bryant and Day method. No exclusion criteria were included in the protocol, to avoid the selection of fit elderly subjects only. Patients with low blood count, abnormal performance status, hepatic, renal, cardiac or pulmonary functions were enrolled. Patients received 4 RP courses (Lenalidomide 25 mg/day for 21 days for 4 cycles, plus Prednisone 50 mg three times/week for 4 months) followed by 6 MPR cycles (Melphalan 2 mg and Prednisone 50 mg three times/week for 6 months plus Lenalido-
mide 10-15 mg/day for 21 days for 6 cycles) and maintenance with RP (Lenalidomide 10 mg/day for 21 days and Prednisone 25 mg three times a week until PD). Two different dose-levels of Lenalidomide were tested in combination with MP: 15 mg (dose-level 1) and 10 mg (dose-level 2). Each cohort included 12 patients, with additional 22 pa-
tients enrolled at dose-level 2. Results. Forty-six patients (median age 75, range 65-88) were enrolled. Forty-four patients were evaluable after a median follow-up of 15.5 months. During RP induction, the most fre-
quent grade 4 hematological adverse events were neutropenia (7%) and anemia (2%), no grade 4 thrombocytopenia was observed. During MPR consolidation no increase in grade 4 adverse events was regis-
tered, incidence of neutropenia was 11%, while no grade 4 anemia and thrombocytopenia were observed. Non-hematological toxicities were more frequent during RP cycles and reduced during MPR cycles (cuta-
naneous rash and infections). Discontinuation rate was higher during in-
duction (14% vs 5%). After RP induction, at least partial response (PR) rate was 78%, at least very good partial response (VGPR) was 16%. During MPR consolidation, PR rate increase to 78%, including 24% of patients who achieved at least a VGPR. CONCLUSION: Induction with RP followed by consolidation with MPR showed a manageable safety profile with a reduced the risk of severe haematological toxicity in unfit elderly myeloma patients.
AN UPDATES ON THE PHASE 1B/2 DOSE-ESCALATION STUDY OF CARFILZOMIB WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE (CRd) IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. Carfilzomib, a novel epoxyketone that specifically and irreversibly inhibits the proteasome, has shown promising single-agent activity in relapsed/refractory multiple myeloma (R/R MM) with a favorable side-effect profile and minimal myelosuppression. Lenalidomide with low-dose dexamethasone (Rd) is a standard of care for patients with relapsed MM. In preclinical studies, lenalidomide sensitized MM to bortezomib, suggesting that combination therapy with these complementary agents may enhance clinical activity. Although the clinical utility of bortezomib/Rd is limited by toxicity, the combination of carfilzomib with lenalidomide and dexamethasone (CRd) may be similarly efficacious and prove to be more tolerable.

Aims. This phase 1b/2 study evaluated the maximum tolerated dose (MTD) of CRd in patients with relapsed or refractory MM. The safety and antitumor activity of the highest dose was further assessed in an expanded cohort.

Methods. Eligible patients included those with relapsed or refractory MM following 1-3 prior therapies (including prior lenalidomide and/or bortezomib). CRd was given on 28-day cycles to 6 cohorts in a 3+3 dose-escalation design. The dosing cycle included carfilzomib on days 1, 2, 8, 9, 15, and 16; lenalidomide on days 1-21, and dexamethasone on days 1, 8, 15, and 22 (Table). An additional 40 patients enrolled in an expansion cohort at the highest dose level. Primary endpoints included safety and establishment of the MTD. Grading of adverse events (AEs) was performed according to NCI CTC v3.0. Additional endpoints included overall response rate (ORR, including partial response or better) as assessed by IMWG criteria, with secondary assessment of clinical benefit response (CBR, minimal response or better) using EBMT criteria. Results. Approximately 75% of the patients enrolled were previously treated with bortezomib. MTD was not reached. Eighty-one patients (50 at the highest dose) were response-evaluable. Initial responses generally occurred within the first 2 cycles and improved with continuing therapy. Responses were observed at all dose levels (Table). As of December 2010, median duration of response has not been reached (>14 mo). Patients who had received 1-3 prior treatment regimens, including high-dose conditioning regimens, had the highest response rates. ORR was 78%, and prolonged administration led to no new or overlapping toxicities. CRd is being directly compared to Rd in patients with relapsed MM in ASPIRE, an ongoing phase 3 open-label, international, multicenter trial.

Table 1.

<table>
<thead>
<tr>
<th>Cohort (1+10)</th>
<th>CRd (mg/m²)</th>
<th>RR (%)</th>
<th>VGPR (%)</th>
<th>SD (%)</th>
<th>PD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort 1</td>
<td>20 or 27</td>
<td>78</td>
<td>9.5</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

Conclusions. The combination of full-dose carfilzomib (20 or 27 mg/m²) with Rd was well-tolerated in MM patients who had received 1-3 prior treatment regimens, including high-dose conditioning regimens. ORR was 78%, and prolonged administration led to no new or overlapping toxicities. CRd is being directly compared to Rd in patients with relapsed MM in ASPIRE, an ongoing phase 3 open-label, international, multicenter trial.

AN ABNORMAL NON-HYPERDIPLOID KARYOTYPE IN MULTIPLE MYELOMA PREDICTS FOR AN ADVERSE OUTCOME AFTER HIGH DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION (HDT/ASCT)

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Background. Despite routine HDT/ASCT in younger patients (pts), a huge heterogeneity in overall survival (OS) outcomes is still observed. This relates to the underlying biological heterogeneity of MM. We evaluate the impact of pre-transplant characteristics and the eventual post-transplant response on the OS of pts who underwent HDT/ASCT in 2 major transplant centers in Singapore. We sought to identify important pts with a history of prior lenalidomide (Lendex) therapy and thalidomide/dexamethasone (TD) before and from 2004 respectively. Bortezomib was available for treatment of relapsed MM and induction treatment of high-risk MM from 2005. All pts received cyclophosphamide 4g/m2 as mobilization followed by peripheral blood stem-cell collection prompted by G-CSF. HDT/ASCT entailed conditioning with melphalan 200mg/m2. Response was defined according to IMWG uniform criteria. Results. The median age of all pts was 55 years. 22%, 43% and 35% of patients presented with ISS stage I, II and III disease respectively. Metaphase cytogenetics detected abnormalities in 44% of pts (hypodiploidy [16%], hyperdiploidy [22%], pseudodiploidy [4%] and near tetraploidy [5%]). For MM subtypes: IgA 15%, IgG 34%, IgD 1%, and light chain MM 7%. Interphase FISH for high-risk markers [deletion17p, (4;14), (14;16)] was positive in 12/52 pts (23%) diagnosed after 2005. For induction, 40% received VAD, while 40% and 20% received TD and bortezomib-based combination respectively. At a median follow-up of 5 years, 65 pts (53%) have been exposed to bortezomib (51% in frontline and 69% at relapse). Overall, the median OS is 8.3 years. Median OS for pts with ISS stage I, II, and III were not reached, 8.1 and 5.1 years respectively (p=0.09). The median OS for pts with diploid, hyperdiploid and non-hyperdiploid karyotype were not reached, 5.1 and 2.6 respectively (p<0.001).

When this analysis was further stratified by bortezomib exposure (non-exposed, frontline or relapse), the impact of cytogenetics was not apparent only among the group of pts who received frontline bortezomib induction. There was a non-significant trend for longer OS among pts attaining at least a very good partial response (sVGPR) (9.5 years vs 5.1 years for sVGPR).
years vs 8.1 years, p = 0.15). On Cox regression multivariate analysis, the presence of a non-hyperdiploid karyotype emerged as the single most adverse prognostic indicator after HDT/ASCT (hazard ratio 4.1, 95% CI 1.2, 14.0, p = 0.03). Conclusion. Our study suggests that while HDT/ASCT may prolong the OS of transplant-eligible pts, it is still unable to overcome adverse cytogenetics detected on conventional metaphase karyotyping. Upfront bortezomib combination prior to HDT/ASCT rather than sequential use at relapse should be considered in this group of pts. Although the attainment of eVGR post-HDT/ASCT has been reported as an important surrogate marker of better prognosis, it does not appear to confer any benefit for pts with a non-hyperdiploid karyotype.

### 0297

**A NOVEL ESTIMATED GFR FORMULA, BASED ON CYSTATIN-C, INDEPENDENTLY PREDICTS FOR SURVIVAL IN PATIENTS WITH NEWLY-DIAGNOSED, SYMPTOMATIC, MULTIPLE MYELOMA: RESULTS IN 157 PATIENTS**

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**Background.** Renal impairment (RI) is a common complication of multiple myeloma (MM). Cystatin-C (Cys-C) is considered as a sensitive marker of glomerular filtration rate (GFR). A recent study in >3,400 patients with chronic kidney disease (CKD) showed that estimating GFR (eGFR) based on serum creatinine(sCr), Cys-C, age, gender and race (eGFR/Cys/Cr) provides the most accurate GFR estimates: eGFR = 177.6 x sCr -0.65 x CysC -0.57 x age -0.20 x (0.82 if female) x (1.11 if black). However, this new formula has not been evaluated in MM. Aim. The aim of this study was to evaluate eGFR/Cys/Cr in newly-diagnosed MM patients, compare it with eGFR assessed by MDRD equation and explore possible correlations with clinical data, including survival. Methods. We studied 157 newly-diagnosed, symptomatic, MM patients (87M/70F, median age 68 years) before any kind of therapy and evaluated both eGFR/Cys/Cr and eGFR/MDRD. Serum Cys-C was determined by par

### 0298

**PATIENT-REPORTED QUALITY OF LIFE (QOL) IN PREVIOUSLY UNTREATED, ELDERLY MULTIPLE MYELOMA (MM) PATIENTS TREATED WITH BORTEZOMIB-BASED REGIMENS: RESULTS FROM THE PHASE 3 UPFRONT STUDY**

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**Background.** The ongoing community-based phase 3b UPFRONT study compares the safety and efficacy of three bortezomib (Vc)-based regimens, Vc-dexamethasone (VcD), Vc-thalidomide-dexamethasone (VcTD), and Vc-thalidomide-prednisone (VcMP), followed by Vc maintenance therapy, in previously untreated, transplant-ineligible MM patients. Aims. To measure changes in patient-reported Qol during Vc-based induction and maintenance. Methods. Patients with symptomatic, measurable MM were randomized (1:1:1) to receive 49 weeks of treatment: eight 5-week induction cycles with VcD, VcTD, or VcMP followed by five 5-week maintenance cycles with single-agent Vc. All patients provided written consent. Adverse events (AEs) were graded by NCI-CTCAE v3.0. Responses were assessed according to IMWG criteria. Patient Qol was recorded using the EORTC QLQ-C30 questionnaire, which assesses global health status, multiple function scores and symptom scores including fatigue, nausea and vomiting, pain, dyspnea, insomnia, appetite loss, constipation, diarrhea, and financial difficulties. Patients completed the questionnaire at baseline, on day 1 of every odd-numbered cycle, at the end-of-treatment visit, and every 12 weeks thereafter. Data were analyzed after 300 patients, 100 per arm, had the opportunity to undergo all 13 treatment cycles. Data imputation was used for patients who died within a year of randomization with missing Qol assessments assigned the worst possible score of zero. Changes in mean global health status scores from baseline, within and between treatment arms, were calculated. Observed data were further analyzed using a linear mixed effect model; a sensitivity analysis using last observation carried forward was also performed. Results. Patient baseline characteristics were well balanced across the treatment arms, as reported previously (Niesvizvy, ASH 2010). Patients received a median of 9 (VcD), 6 (VcTD), and 7 (VcMP) treatment cycles; 56%, 33%, and 43% of patients, respectively, received Vc maintenance. After 13 cycles, re-
response rates were 71% (VcD), 79% (VcTD), and 73% (VcMP); ≥VGPR rates were 39%, 47% and 44%, respectively. Rates of grade ≥3 AEs after 13 cycles were 74% (VcD), 86% (VcTD), and 80% (VcMP); serious AEs were highest for VcTD (61% vs 57% VcD and 51% VcMP), as was the rate of discontinuation due to AEs (41% vs 29% VcD and 35% VcMP). QoL assessments were available at baseline and ≥1 post-baseline time point for 80% (VcD), 67% (VcTD), and 80% (VcMP) of patients. The observed data indicate that all treatment groups experienced a downward trend in mean global health status score until cycle 7 or 8, followed by an increase or stabilization by the end of treatment (Figure); there were no differences between treatment arms. Symptom scores changed very little during induction with all Vc-based regimens, with moderate improvements seen during maintenance, except for nausea/vomiting and diarrhea. The trends to decreased QoL score seen during Vc-based induction may reflect the onset of treatment-associated AEs. Post-induction increases in QoL may reflect the positive impact of achieving a response and the limited toxicity profile associated with Vc maintenance. Patients continue to be followed for QoL assessment and long-term outcomes.

**0299**

**ADVERSE CYTOGENETICS DO NOT AFFECT RESPONSE RATE OR DURATION IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (R/R MM) TREATED WITH SINGLE-AGENT CARFILZOMIB**

S Siegel,1 S Singhal,3 T Martin,1 M Wang,4 R Vij,6 A Jakubowiak,6 S Loni,2 V Kukreti,7 N Bahlis,1 A Chanen-Khan,10 M Alsina,11 G Somlo,12 F Buadi,13 F Reu,14 K Song,1 L Kunkel,16 A Wong,17 M Vallone,17 R Ozolska,8 A Stewart,13 S Jagannath,18 Multiple Myeloma19 et al.

**Aims.** The objective of this analysis was to evaluate the influence of cytogenetics in a large phase 2b study (PX-171-008-A1) of single-agent carfilzomib in patients with R/R MM. **Methods.** 229 of 266 patients enrolled (86%) were response-evaluable and had available metaphase cytogenetics and/or fluorescence in situ hybridization (FISH) analyses for adverse cytogenetics defined per mSMART criteria (hypodiploidy, chromosomes 13 deletions, del17p13, t(4;14), and t(14;16) chromosomal abnormalities). Metaphase data were available for 200 patients (75%), FISH data for 205 patients (77%). All patients received carfilzomib at 20 mg/m² IV on days 1, 2, 8, 9, 15, and 16 in a 28-day cycle (C) followed by an increase or stabilization by the end of treatment (Figure); there were no differences between treatment arms. Symptom scores changed very little during induction with all Vc-based regimens, with moderate improvements seen during maintenance, except for nausea/vomiting and diarrhea. The trends to decreased QoL score seen during Vc-based induction may reflect the onset of treatment-associated AEs. Post-induction increases in QoL may reflect the positive impact of achieving a response and the limited toxicity profile associated with Vc maintenance. Patients continue to be followed for QoL assessment and long-term outcomes.

**Carfilzomib** is a novel, highly selective epoxysoukone proteasome inhibitor that produces durable single-agent activity in patients with R/R MM. Aims. The objective of this analysis was to evaluate the influence of cytogenetics in a large phase 2b study (PX-171-008-A1) of single-agent carfilzomib in patients with R/R MM. **Methods.** 229 of 266 patients enrolled (86%) were response-evaluable and had available metaphase cytogenetics and/or fluorescence in situ hybridization (FISH) analyses for adverse cytogenetics defined per mSMART criteria (hypodiploidy, chromosomes 13 deletions, del17p13, t(4;14), and t(14;16) chromosomal abnormalities). Metaphase data were available for 200 patients (75%), FISH data for 205 patients (77%). All patients received carfilzomib at 20 mg/m² IV on days 1, 2, 8, 9, 15, and 16 in a 28-day cycle (C) followed by an increase or stabilization by the end of treatment (Figure); there were no differences between treatment arms. Symptom scores changed very little during induction with all Vc-based regimens, with moderate improvements seen during maintenance, except for nausea/vomiting and diarrhea. The trends to decreased QoL score seen during Vc-based induction may reflect the onset of treatment-associated AEs. Post-induction increases in QoL may reflect the positive impact of achieving a response and the limited toxicity profile associated with Vc maintenance. Patients continue to be followed for QoL assessment and long-term outcomes.

**Table 1.**
MULTIPLE MYELOMA: PHASE 1 DOSE-ESCALATION STUDIES

M Wang,1 D Siegel,1 J Kaufman,2 A Jakubowiak,3 A Stewart,4 S Jagannath,5 V Kukreti,5 K McDonagh,6 M Alsin,7 N Bahlis,8 A Belin9, F Rev,10 N Gabrail,11 J Matyi,12 S Hwang,12 P Lee,13 C Shustik,15 K Doner,18 R Orlofski,1 L Kunkel,19 A Wong,20 R Vij,21 Multiple Myeloma22

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Background. The selective, epoxysketone proteasome inhibitor carfilzomib produces potent, sustained proteasome inhibition while lacking many of the off-target activities associated with bortezomib. Carfilzomib produces durable single-agent activity in patients with relapsed/refractory multiple myeloma (R/R MM) who have received multiple prior therapies, as well as in patients with advanced-stage disease or significant comorbidities. PX-171-004 is an ongoing phase 2 study of single-agent carfilzomib in patients with relapsed or refractory MM following 1-3 prior therapies. Aims. Here we present updated data on the bortezomib-naive patients treated on study. Methods. Patients received either 20 mg/m² for all treatment cycles (Cohort 1) or a step-up dose-escalating regimen of 20 mg/m² for Cycle 1 and 27 mg/m² for all treatment cycles thereafter (Cohort 2). Carfilzomib was administered on days 1, 2, 8, 9, 15, and 16 of every 28-day cycle, for a maximum of 12 cycles. The primary endpoint was the overall response rate (ORR; CR + VGPR + PR) determined according to the International Myeloma Working Group Uniform Response Criteria. Secondary endpoints included the clinical benefit response (CBR; ORR + MR) rate, time to progression (TTP), duration of response (DOR), and safety. Results. 123 of 125 enrolled bortezomib-naive patients were evaluable for response. Prior therapies included thalidomide (58%), lenalidomide (59%), alkylating agents (82%), and stem cell transplant (73%). 44 patients had disease refractory to the most recent therapy. A median TTP of 8.3 months and a median DOR of 13.1 months were observed for Cohort 1. The median TTP and DOR for Cohort 2 have not been reached. The most common treatment-emergent adverse events (AEs), regardless of relationship to carfilzomib, were fatigue (60%), nausea (45%), anemia (40%), and dyspnea (26%). These were primarily ≥ Grade 2 in severity. The most common Grade 3/4 AEs were anemia (15%), lymphopenia (13%), pneumonia (13%), neutropenia (12%), and thrombocytopenia (11%). Treatment-emergent peripheral neuropathy (PN) was infrequent (18%) and mild. Only 1 case of Grade 3 PN (0.8%) was observed. Overall, 49 patients (40%) completed 12 cycles (1-12+), and 32 (26%) continued to receive carfilzomib therapy on extension protocol PX-171-010, including 11 patients from bortezomib-naive Cohort 1 and 27 patients from bortezomib-naive Cohort 2. Significantly, no cumulative toxicities have been noted. Conclusions. Single-agent carfilzomib achieves high response rates in bortezomib-naive patients with relapsed myeloma, with minimal neuropathy. At the recommended phase 3 dose of carfilzomib, the 53% ORR is noteworthy for a single-agent regimen for patients with myeloma who had received 1-3 prior regimens for MM. Moreover, prolonged carfilzomib treatment is well-tolerated, with ~40% of patients completing 12 cycles and 29% continuing treatment beyond 1 year.

0301
EVALUATION OF TWICE-WEEKLY AND WEEKLY DOSING OF THE INVESTIGATIONAL AGENT MLN9708, AN ORAL PROTEASOME INHIBITOR, IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: PHASE 1 DOSE-ESCALATION STUDIES

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Background. MLN9708 is an investigational, orally bioavailable, potent, reversible, and specific 20S proteasome inhibitor. MLN9708 is active in solid tumor and hematologic xenograft models. IV and oral formulations are in phase 1 trials. Aim. Two studies of oral MLN9708 are ongoing to assess its safety, maximum tolerated dose (MTD), pharmacokinetics (PK), and anti-tumor activity in patients with relapsed/refractory multiple myeloma (MM). Methods. Adults with MM after ≥2 prior therapies, which must have included bortezomib, thalidomide/lenalidomide, and corticosteroids, were eligible. Patients provided informed consent. Patients received MLN9708 on days 1, 4, 8, 11 of 21-day cycles (twice-weekly [TW]) or days 1, 8, 15 of 28-day cycles (weekly [W]). Dose-escalation proceeded from 0.24 mg/m² using a standard 3+3 schema based on the occurrence of dose-limiting toxicities (DLTs) in cycle 1. Adverse events (AEs) were graded by NCI-CTCAE v3. PK samples were collected after single and multiple doses for both schedules. Response was assessed by modified EBMT criteria. Results. To date, 26 patients (16 male, median age 64.6 years [range 50-83]) have received MLN9708 TW at 0.24, 0.48, 0.8, 1.2 (each n=3), 1.68 (n=4), 2.0 (n=7), and 2.23 mg/m² (n=4), and 22 patients (12 male, median age 63.5 years [range 40-76]) have received MLN9708 W at 0.24, 0.48, 0.8, 1.2 (each n=3), 1.68 (n=4), 2.0, and 2.97 mg/m² (each n=5). Patients received medians of 4 cycles (range 1-12+) on the TW and 2 cycles (range 1-8+) on the W schedules; 9 and 8 patients remain on treatment, respectively. Safety data are shown in the Table. The MTD for MLN9708 TW was established as 2.0 mg/m² and MTD on the W schedule has not yet been reached. All-cause grade 3/4 AEs on the TW schedule included thrombocytopenia (n=6), neutropenia (n=2), and non-cardiac chest pain (n=2) and on the W schedule included...
A SUMMARY OF SAFETY AND EFFICACY DATA ACHIEVED WITH LONG-TERM CARFILZOMIB (CFZ) TREATMENT IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA (R/R MM)

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Background. Carfilzomib (CFZ) is a novel, highly selective proteasome inhibitor that differs from Bortezomib both structurally and mechanistically. CFZ does not cause cumulative toxicity (including neurotoxicity) in long-term (6-9 month) chronic animal toxicology studies. Phase 2 clinical trials of single-agent CFZ in MM demonstrated durable responses in patients with relapsed and refractory (RR) multiple myeloma (MM), including patients with pre-existing peripheral neuropathy and subclinical renal dysfunction. Here we report on the updated clinical experience with long-term treatment (>12 cycles) with carfilzomib in patients with MM. Methods. Patients treated in phase 1 or 2 MM trials were eligible to enroll in an extension study after 12 cycles. Patients initially received carfilzomib on Days 1, 2, 8, 9, 15, and 16 of every 28-day cycle. The dosing frequency could be reduced to alternate weeks (ie, Days 1, 2, 15, and 16). Dose increases up to a maximum of 56 mg/m² were also permitted. Depending upon the time of administration from the patients’ original study, IV carfilzomib was administered over a period of either 10 minutes or 30 minutes. In selected patients, a second anti-myeloma agent was added. Patients continued treatment until evidence of progressive disease or unacceptable toxicity, or individual withdrawal of consent. Results. As of 31 January 2011, 78 patients with MM had enrolled. The relative proportion of patients rolling over was highest from the 2 phase 2 trials: 29/266 patients (11%) from 008-A1 (R/R MM pts) and 38/129 patients (29%) from 004 (bortezomib-naïve, 1-3 prior regimens). Sixty-one MM patients (78%) remain on study and are receiving a median dose of 27 mg/m² (range: 15-56 mg/m²). The median total duration of treatment (original study + extension study) is 18 months. 29 patients remain on their original dosing schedule, and 21 patients are receiving the intermittent dosing schedule. The longest total duration of treatment is >30 months. 18 patients are study due to progressive disease, 1 patient was removed at the investigator’s discretion, and 1 patient withdrew consent. 2 patients had dose reductions due to toxicity, and there have been no withdrawals due to toxicity. 15 patients had their doses of carfilzomib increased, 2 patients added lenalidomide, and 4 patients added 40 mg/wk dexamethasone. Clinically significant cumulative toxicities were not observed. Conclusions. CFZ can be safely administered to patients with MM for extended therapy using the either the original dosing schedule or an intermittent schedule. The maintenance carfilzomib treatment regimen sustains disease control and provides excellent long-term tolerability.
REAL-LIFE DATA ON THE CURRENT APPROACH TO OLDER PATIENTS WITH MULTIPLE MYELOMA

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Background. The combination melphalan-prednisone (MP) has represented the standard of care for older patients with multiple myeloma (MM). The introduction of new agents has challenged the role of MP and led to new standards of care, even in aged individuals. All randomized studies comparing MP with MP plus thalidomide (MPT) showed advantage in progression free survival for MPT; in addition, in two of these studies a survival advantage was recorded. More recently, superiority of MPV over MP was also demonstrated for the combination of MP plus bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the bortezomib (MPV). Accordingly, the combination of MP with a new agent is the current standard of care in MM of the elderly. While in the

METHODS. From 2008 to 2010, 257 patients over 65 years were diagnosed at our institutions. The median age was 74 years and all of them had symptomatic disease requiring treatment. Twenty-five patients (12%), all aged 66-74 years, who received autologous stem cell transplantation were excluded from the analysis. Among the remaining 212, 47 patients received MP (22%), 102 MPT (48%) and 65 (30%) MPV. Among MPT and MPV patients, 66 % were accrued into prospective clinical trials; conversely, the MP subgroup was judged as eligible for any trial (p >0.001). The median age was 77 years for the MP patient subgroup, as opposed to 71 for MPV and 75 for MPT; the difference was statistically significant between MP and MPV (p=0.004) as well as between MP and MPT (p=0.007), while it was not significant between MPV and MPT (p=0.14). The median number of comorbidities requiring specific treatment was 3 (range 1-5) in the MP subgroup as opposed to 1 in the MPT (range 0-1) and 0 (range 0-1) in the MPV subsets. Once again, differences between MP and MPT (p=0.03) and between MP and MPV (p=0.01) were statistically significant. The main criteria for the selection between MPT and MPV were distance from hospital, travel and the period of observation (more patients received MPV after the registration as first line). Conclusions. We conclude that 22% of older MM patients receive suboptimal therapy, i.e. the old MP combination. More advanced median age and number of severe comorbidities requiring specific treatment account for the therapeutic selection. Among patients treated with current standard of care, the choice between MPV and MPT is strictly related to the ability of patients to travel as well as to distance from the hematological institution. Of note, most data refer to specialized hematological institutions; it is conceivable that in non hematological wards, the percentage of exclusion from the current standard of care can be higher.
chronic hemodialysis and shortened survival. Reversal of renal impairment requires immediate installment of effective, anti-myeloma therapy and rapid reduction of the pathogenic light chains. *Aims.* To prospectively evaluate the efficacy of Lenalidomide (L)-Dexamethasone (D) in restoring renal function and tumor control in patients with light chain-induced acute renal failure (LC-ARF). *Patients and Methods.* 21 patients with LC-ARF as formerly defined (JCO 2010) have been identified for this trial and informed consent was obtained from all patients; 1 patient died before first study medication and for 2 patients data are not available as yet. Lenalidomide was given from d 1-21 with dose adaptation according to GFR. Dexamethasone 40 mg was administered on d 1-4, 9-12, 17-20 during cycle 1; thereafter ttx-week. Cycles were repeated every 28 days. *Results.* Baseline characteristics are available for 18 pts at present: Median age: 68 years (range: 47-87 years), male/female: 8/10, 17 patients presented with ISS stage III (1 data missing). 17 (94.4%) presented with de novo MM and 1 (5.5%) with previously treated, but relapsing disease; median GFR 21.2 ml/min (range 6.1 - 27.6 ml/min). ECOG performance status was 0 in 6 patients, 1 in 4 pts, 2 in 5 pts, 3 in 1 pt and 4 in 2 pts. Presently, 12 patients are evaluable for response (completed ≥2 cycles and fully documented). 6 patients completed all 9 cycles, 2 patients 6 cycles and 1 patient each completed 7, 5, 3 and 2 cycles. nCR was achieved in 7 (58.3%), VGPR in 1 (8.3%), PR in 1 (8.3%), and MR in 3 (25.0%) patients, respectively. yielding a nCR/PR rate of 75%. Median time to tumor response was 129 days. Renal response was assessed as formerly defined (ICO 2010), 2 patients achieved CR/RENAL and 6 PR/MR/RENAL, respectively, yielding an OR/RENAL in 8 (61.5%) of the evaluable pts. Median time to best renal response was 147 days. 3 of 8 dialysis dependent patients became dialysis independent. Median GFR of evaluable patients increased from 14.7 (range 6.1 - 27.6 ml/min) at baseline to a median best GFR of 28.9 ml/min (range 11.3 - 74.0 ml/min). In 7 patients with nCR median GFR improved from 9.4 to 30.6 (11.3-74 ml/min). In 5 patients with VGPR/PR/MR median GFR increased from 14.7 to 26.0 (16.8 - 40.7 ml/min). Full documentation of adverse events is presently available in 16 patients. 2 patients died due to infection (12.5%). Grade 3/4 anemia, thrombopenia and leucopenia, were seen in 9 (56.2%), 2 (12.5%), and 1 (6.2%) patients, respectively. Other common grade 3/4 toxicities were infection 5 (31.2%), and cardiac dysfunction 3 (18.8%). Exanthena, pulmonary embolism, macula edema, and fatigue were seen in 1 patient each (6.2%). Conclusions. LD showed significant antmyeloma activity and improved renal function in 62% of this high risk population. The LD regimen with the dose of lenalidomide adjusted according to GFR was well tolerated. Updated results will be presented.

### 0307

**BORTezomIB-BaseD therapy overcomes the prognOSTIC ROLE of ISS SCORE in MULTIPLE MYELOMA patients not eligible for transPLANT**

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**Background.** Novel agents had considerably changed the outcome in multiple myeloma (MM) patients not eligible for transplant. The achievement of a CR correlates with long-term progression free survival (PFS) even in this setting. PFS could be significantly variable in patients with the same type of response. Among baseline prognostic factors, ISS score 3/2/1 was associated with better response and survival in patients treated with novel agents. *Aims.* We explore the role of ISS in predicting the length of progression free survival (PFS) in MM patients not eligible for transplant stratified according to response. *Methods.* We retrospectively reviewed the records of 511 patients enrolled in the controlled multicenter randomized phase III GIMEMA trial VMPT-VT vs VMP (Palumbo et al, JCO 28:5101-5109, 2010). Patients were randomly assigned to received 9 VMP cycle (bortezomib+melphalan+prednisone-thalidomide) followed by VT maintenance (bortezomib-thalidomide) for 2 years (or until progression) (VMPT-VT arm, 254 patients) or 9 cycle of VMP (bortezomib+melphalan+prednisone) without maintenance (VMP arm, 257 patients). The study was performed according to the Declaration of Helsinki, and approved by the ethics committee of each participating institution. Response to therapy was defined according to EBM7 criteria. Patients were considered responsive (ORR) when obtaining at least a PR. Results. ISS distribution was balanced in the two arms (p=0.40) with 19%/39%/25% and 22%/34%/22% of patients with ISS 3/2/1 in VMPT-VT and VMP arm respectively. ORR was 89% (CR 38%, VGPR 21%, PR 30%) in VMPT-VT arm vs 81% (CR 24%, VGPR 26%, PR 31%) in VMP arm (p=0.01). Longer PFS was observed in patients obtaining a CR with no statistical difference between patients with VGPR and PR both in VMPT-VT and VMP arm (p=0.35 and p=0.6 respectively). The prognostic role of ISS on PFS was evaluted in the whole population and after stratification according to response and study arm. In univariate analysis ISS showed no significant impact on PFS (p=0.9). In patients reaching a CR, ISS showed no significant correlation with PFS in both treatment arm (p=0.8 and p=0.6 in VMPT-VT and VMP arm respectively). In patients with less than a CR (VGPR/PR) low ISS (1) was associated with better PFS, only in patients in VMP arm (p=0.04), without significant correlation for patients with similar response enrolled in VMPT-VT arm (p=0.18). Conclusions. In MM patients not eligible to transplant, the prognostic role of ISS is overcome by bortezomib-based treatment. In patients with less than a CR, ISS have a negative prognostic impact on PFS only in those who do not receive a maintenance.

### 0308

**LONG-TERM FOLLOW-UP IN PX-171-003-A1, AN OPEN-LABEL, SINGLE-ARM PHASE 2 STUDY OF CARfilZOBIM in PATIENTS WITH RELAPSED AND refractory MULTIPLE MYELOMA (R/R MM): ANALYSIS BY SUBGROUPS OF INTEREST**

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**Background.** Carfilzomib is a novel, highly selective epoxyketone proteasome inhibitor in development for treatment of MM. Single-agent carfilzomib has demonstrated durable activity in patients with R/R MM in phase 1 and 2 studies. The study was performed according to the Declaration of Helsinki, and approved by the ethics committee of each participating institution. Response to therapy was defined according to EBM7 criteria. Patients were considered responsive (ORR) when obtaining at least a PR. Results. ISS distribution was balanced in the two arms (p=0.40) with 19%/39%/25% and 22%/34%/22% of patients with ISS 3/2/1 in VMPT-VT and VMP arm respectively. ORR was 89% (CR 38%, VGPR 21%, PR 30%) in VMPT-VT arm vs 81% (CR 24%, VGPR 26%, PR 31%) in VMP arm (p=0.01). Longer PFS was observed in patients obtaining a CR with no statistical difference between patients with VGPR and PR both in VMPT-VT and VMP arm (p=0.6 and p=0.5 respectively). The prognostic role of ISS on PFS was evaluated in the whole population and after stratification according to response and study arm. In univariate analysis ISS showed no significant impact on PFS (p=0.9). In patients reaching a CR, ISS showed no significant correlation with PFS in both treatment arm (p=0.3 and p=0.06 in VMPT-VT and VMP arm respectively). In patients with less than a CR (VGPR/PR) low ISS (1) was associated with better PFS, only in patients in VMP arm (p=0.01), without significant correlation for patients with similar response enrolled in VMPT-VT arm (p=0.18). Conclusions. In MM patients not eligible to transplant, the prognostic role of ISS is overcome by bortezomib-based treatment. In patients with less than a CR, ISS have a negative prognostic impact on PFS only in those who do not receive a maintenance.
Table 1.

Table 1

Baseline characteristic

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<th>Characteristic</th>
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Results from a multicentre study

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN FIRST LINE MULTIPLE MYELOMA: DOES IT STILL EXIST?

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Material and Methods.

This study concerned MM patients (pts) prospectively allocated to receive a tandem auto-allo-HSCT for being of bad prognosis. They received RIC followed by allo-HSCT after achieving at least a PR to auto-HSCT. RIC regimen in majority combined Fludara, 30mg/m²/d (d-5[ARROWRIGHT]d-1), Busilvex 4.3mg/kg/d (d-4, d-3) and ATG 2.5mg/kg/d (d-2, d-1). This analysis included 25 pts, 18 males and 7 females, median age 51 years [28-67], there were 15 IgG, 6 IgA and 4 light chains MM, 14 pts had del17, 7 had del1 and 17 had high level of beta2µ; 7 pts had the 3 factors combined and 6 pts had 2 factors combined. For induction therapy, 16 pts received VAD and 9 patients received Veld. Pts received auto-HSCT after a median time of 5.5 months [3.6-15.3] from diagnosis. All pts were in PR after auto-HSCT and before allo-HSCT. Allografts came from 16 identical siblings, 8 matched (10/10) and 1 mismatched (9/10) unrelated donors. Results. At Day 90, 10 pts were in CR, 15<CR among them 9 received Velcade, 6 received other treatments including DLI. There were 8 acute GVHD (2 grade II and 1 grade III) and 11 chronic GVHD (8 limb. and 3 ext.). At the last follow-up, 10 pts were in durable CR1 post allo-HSCT. After a median follow-up of 40 months [3-125], the median OS was not reached with a 6 years probability of 70% [53-85], the median PFS was 36 months [16-125], with a 6 years probability of 45% [29-71], TRM was 8% at 2 years and reached 12% at 4 years. Conclusion. According to these very promising results, we should reconsider the allo-HSCT as a first line treatment for MM especially for pts with poor prognostic factors. The development of novel reduced-intensity preparative regimens, pre- and post-transplantation strategies enhancing the graft-versus-myeloma effect are the important key issues for the future. RIC Allo-HSCT should be optimized rather than replaced.

0310

EVALUATION OF 18F-FDG PET/ CT AND 99MTc-MIBI SCINTIGRAPHY IN PATIENTS WITH MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE: COMPARISON OF METHODS

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Newer imaging modalities, such as 18F-FDG PET/ CT and 99mTc-MIBI scintigraphy, have been recently introduced to assess the activity and extent of disease in patients with multiple myeloma (MM) and gammapathy of undetermined significance (MGUS). The aim of our study was to compare the impact of these imaging modalities in the evaluation of MM and MGUS patients. Materials and Methods. A total of 101 patients with MM (81 patients) and MGUS (20 patients) were enrolled in the study (21 newly diagnosed and 44 relapsed patients with symptomatic MM, 16 with asymptomatic MM and 20 with MGUS). All patients were without therapy and underwent 18F-FDG PET/ CT and 99mTc-MIBI scintigraphy within a maximum interval of 14 days. The scans were classified as normal (N), diffuse (D), and focal or combined (F-FD) pattern. Results. There was no significant difference in the detection of newly diagnosed MM and relapsed patients between the compared methods. 18F-FDG PET/ CT performed better than 99mTc-MIBI scintigraphy in the detection of focal lesions (p < 0.0059), whereas 99mTc-MIBI scintigraphy was superior in the visualization of diffuse use disease (p = 0.042). 18F-FDG PET/ CT visualised significantly more focal lesions than 99mTc-MIBI scintigraphy (p = 0.002), both generally in the cohort and when comparing the number of focal lesions per patient. Both the imaging modalities singly or in combination fulfilled the subsequent clinical management in 17% of patients. In our study, 18F-FDG PET/ CT predicted asymptomatic MM and MGUS transormatio ninto more aggressive forms with the necessity to start therapy more often than 99mTc-MIBI scintigraphy. Conclusion. 18F-FDG PET/ CT appeared to be a better imaging technique than 99mTc-MIBI scintigraphy in the detection of focal lesions in patients with symptomatic MM.99mTc-MIBI scintigraphy was superior in the visualization of diffuse use disease. On the other hand, despite its limited capacity in detecting focal lesions, 99mTc-MIBI scintigraphy still remains the most rapid and inexpensive technique for whole-body evaluation and may be an alternative option when a PET/ CT facility is not available.
**0311**

**EFFECT OF TREATMENT WITH LENA / DEXA OF ASYMPTOMATIC MULTIPLE MYELOMA AT HIGH RISK OF PROGRESSION ON BONE REMODELING MARKERS AND CYTOKINES RELATED TO BONE DISEASE**

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**Background.** We know the changes in bone remodeling markers and cytokines related to bone disease in Multiple Myeloma (MM) patients treated with different drugs (chemotherapy, Bortezomib, Lenalidomide, Thalidomide, ...), but so far this subject is not studied in smoldering MM (sMM) patients. Aims: To analyze sequential changes of serum bone parameters in sMM at high risk of progression treated with Lenalidomide and Dexamethasone. **Material and Methods.** Phase III trial has been conducted by the Spanish Myeloma Group (GEM), in which sMM patients at high-risk of progression were randomized to receive Len-dex (9 cycles) in induction followed by a maintenance phase with Len alone vs. no treatment in order to evaluate whether the early treatment prolongs the time to progression (TTP) to symptomatic disease. In the serum of these patients, we analyzed one bone resorption marker (C-terminal telopeptide (CTX)), one bone formation marker (bone alkaline phosphatase (BAP)) and some cytokines related to bone disease pathogenesis in MM (Macrophage inflammatory protein 1-alpha (MIP-1alpha), sRANKL, osteoprotegerin (OPG) and DKK1) before the start of treatment, after 3 and 9 cycles. **Results.** We analyzed these parameters in 53 patients in the treatment group who achieved at least partial remission, 20 of them underwent sequential analysis of the parameters above. The only two parameters that showed significant sequential changes in this subset of patients were DKK1 and OPG. In the case of DKK1, a significant decrease of the same after 3 months of treatment (mean ± SD: 55.46 ± 26.9 (at baseline) to 20.80 ± 13.21 (after 3 cycles) (p = 0.007)), which stabilized after 9 cycles (22.17 ± 13.06). The OPG showed late decrease (6.28 ± 3.07 (at baseline) to 5.10 ± 2.87 (after 9 cycles) (p = 0.013). **Summary.** The decrease in DKK1 has been described in patients with MM. It has been postulated to be due to the reduction of malignant plasma cells, which would also explain the decline in our patients after 3 cycles of treatment. The stabilization of DKK1 levels after 9 cycles could be explained because in patients with low tumor mass, tumor control could be done especially in the first cycles. The OPG is produced by osteoblasts and mesenchymal cells, so the late fall of OPG observed in our study would imply a decrease in the synthesis and osteoblastic activity, which could be due to the action of Dexamethasone. **Acknowledgment.** Granted by Celgene Laboratories.

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**0312**

**NOVEL AGENTS ARE BENEFICIAL FOR REAL LIFE PATIENTS WITH MULTIPLE MYELOMA NOT ELIGIBLE FOR HIGH DOSE TREATMENT**

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**Background.** High dose treatment (HDT) in Multiple Myeloma (MM) has been shown to be superior to standard chemotherapy. However, a large amount of patients are not eligible for HDT. Evaluating real life outcomes in those patients is of high interest and may identify prognostic factors. **Aim.** Evaluate real life responses, time to progression (TTP), time to next therapy (TTNT) and overall survival (OS) and prognostic power of patient variables in real life non-HDT patients with MM. **Methods.** All non-HDT patients, n=269, diagnosed with MM between Jan 2000 and Jul 2010 at Karolinska Huddinge and Jan 2005 and Jul 2010 at Karolinska Solna were included. Near complete response (nCR) was defined as an immeasurable M-protein by standard electrophoresis. Very good partial response (VGPR), partial response (PR), no response (NR) and TTNT were defined according to IMWG criteria. Standard statistical methods were used. **Results.** The median age was 72 years, 60% 51% male. Baseline creatinine was 98 µmol/L, albumin 33 g/L, hemoglobin 110 g/L and β2-microglobulin 3.8 mg/L. The median number of treatment lines was 2. 58% of the patients were given at least 2 treatment lines and only 34% 3 or more. The response distribution nCR/VGPR/PR/NR was 15/16/49/56% in 1st line, 15/29/50/55 in 2nd line, nCR in the 1st line implied a 45% probability to receive a > VGPR and 26% probability to receive an nCR in 2nd line. NR in the 1st line implied a 55% probability to receive a NR in the 2nd line. **Logistic regression analysis shows** that the patients receiving novel agents (Bortezomib, Lenalidomide, Thalidomide) in 1st line had a higher probability of achieving nCR. Baseline creatinine values, β2-microglobulin and most likely also albumin and hemoglobin, seem to be of importance for the response. The median TTP/TTNT was 248/301 days in the 1st line 176/187 in 2nd line and 180/210 in 3rd line. There was a significant trend of increasing TTP/TTNT in 1st line depending on the increased depth of the response with TTP/TTNT for nCR of 423/481 days, VGPR 269/376 days, PR 269/376 days and NR 190/176. Kaplan-Meier analysis shows that the use of novel agents in 1st line predicted a longer TTP. Median OS was 3.6 years 95% CI [2.9;4.4] with 44% censored. The median OS for patients with 1st line best response nCR was 4.9 years, VGPR 3.9 years, PR 4.0 years and NR 1.9 years. There is a correlation between TTP in 1st line and increased OS. Kaplan-Meier analysis shows that use of novel agents in the 1st line predicted a longer OS, median 5.1 years vs. 2.8 years. Baseline patient variables that showed to have significant importance for OS were creatinine, albumin, hemoglobin and β2-microglobulin. **Summary.** Patients receiving 2nd and 3rd line treatment were declining rapidly. Receiving a nCR in 1st line seems to be very important. Receiving a VGPR or PR seems to give similar results, less good than nCR but better than NR. Variables of importance are creatinine, albumin and hemoglobin. The use of novel agents improves response, TTP and OS.

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**0313**

**A COMPARISON OF REDUCED-INTENSITY CONDITIONING FOR ALLOGRAFTING (RIC) FOLLOWING AUTOGRAPHING (ASCT) VERSUS DOUBLE AUTOGRAPHING FOR NEWLY MULTIPLE MYELOMA (MM) PATIENTS. TEN YEARS EXPERIENCE**

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**Background.** Multiple Myeloma (MM) is the most frequent indication for autografting (ASCT). ASCT represents the most effective palliation for these patients. Even the double ASCT where there is demonstrated good long-term control in a minority of patients do not appear to result in cure of disease. Myeloablative AlloSCT is penalized by excessive transplant-related mortality (TRM) and toxicity. The introduction of reduced-intensity conditioning for autologation (RIC) has renewed interest in the use of AlloSCT for MM. **Aims.** Recent studies have reported encouraging results with tandem ASCT followed shortly thereafter by RICT in MM patients as compared to ASCT or myeloablative AlloSCT alone. Here we present an update at 10 years of the results.
achieved in our Unit in patients receiving double ASCT compared to patients receiving tandem ASCT/RICT. Methods. We enrolled 132 consecutive patients 65 years of age or younger with stage II or III myeloma. One hundred seven patients had siblings and 95 patients and their siblings underwent HLA typing. Thirty-four of them had an HLA-identical sibling. All patients were initially treated with induction chemotherapy consisting of 3–4 courses of VAD regimen or modifications of VAD regimen. Soon after, peripheral blood stem cells (PBSC) were collected after 3–4 gr of cyclophosphamide per square meter of body surface area. G-CSF was given 4–5 days after chemotherapy. Daily aphereses was continued until at least >8x10^6 CD34 cells/kg were collected. After the first ASCT, patients who had an available HLA-identical sibling donor were offered RICT. Patients without an HLA-identical sibling donor underwent a second ASCT. The conditioning regimen of ASCT-1 and ASCT-2 consisted of melphalan 200 mg/m² infused over 30 minutes. The RICT consisted of fludarabine 30 mg/m² daily for 3 days and TBI (2 Gy or melphanal 70 mg/m²). Graft-versus-host prophylaxis consisted of cyclosporine A and short-course methotrexate. Results. The rate of CR was significantly higher in RICT arm (RICT: 54.1%; ASCT: 28.5%). At December 2010 9 (57.5%) of 24 patients who received RICT and 4 (11.1%) of 35 patients who received double ASCT were in continuous CR after a median of 57 months (range, 14-88 months) and 65 months (range, 28-70 months), respectively. Thirteen (54.1%) patients in the ASCT/RICT group and 12 (34.2%) in the double ASCT were alive at a median of 104 months (range, 45-124 months) and 65 months, respectively. The cumulative incidence rate of acute and chronic GVHD after RICT was 41% and 54%. In the RICT arm, 12 patients died of: extensive chronic GVHD (4 patients), progressive disease, (5 patients) and cGVHD and infections (3 patients). At December 2010, 15 patients in RICT and 12 patients in double ASCT are alive after a median of 104 mo. (range, 45-124) and 62 mo. (range, 20-118), respectively. Conclusions. This update retrospective analyses suggests that ASCT/RICT may be able to reduce the incidence of disease progression.

0314 A PHASE IB STUDY OF ORAL PANOBINOSTAT PLUS INTRAVENOUS BORTEZOMIB IN RELAPSED OR REFRACTORY MULTIPLE MYELOMA: UPDATED RESULTS AT THE EXPANSION PHASE

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Background. Panobinostat is an oral pan-deacetylase inhibitor (pan-DACI) that increases acetylation of proteins involved in multiple oncopathic pathways. In preclinical studies, panobinostat has demonstrated synergistic activity in combination with bortezomib which may occur through inhibition of the aggresome and proteasome pathways causing an accumulation of intracellular misfolded cytotoxic proteins leading to multiple myeloma (MM) cell apoptosis. Aims. This phase Ib study sought to identify the maximum tolerated dose (MTD) of the combination of panobinostat and bortezomib in patients with relapsed or refractory MM. Safety and efficacy was further evaluated in an expansion phase using a modified dosing schedule of panobinostat at the MTD of the combination. Methods. This study completed enrollment (N = 62) in December 2010 with 47 patients in the dose escalation phase and 15 patients in the dose expansion phase. In the dose escalation phase, panobinostat was administered orally thrice weekly in combination with bortezomib (intravenous, days 1, 4, 8, 11) in 21-day cycles. Dexamethasone was to be added in case of suboptimal response from cycle 2 onwards (n = 16 patients). The MTD was determined to be 20 mg panobinostat plus 1.3 mg/m² bortezomib. In the expansion phase, 15 patients received the MTD of panobinostat plus bortezomib, with a modified panobinostat schedule (2 weeks on, 1 week off) and dexamethasone was introduced for all patients, from cycle 2 onwards allowing time for pharmacokinetic analysis of panobinostat and of bortezomib before as well as after introduction of dexamethasone. This dosing schedule was evaluated in an effort to increase tolerability and maximize therapy duration and is identical to the schedule in the ongoing phase II and III PANORAMA trials. Results. Patients in the dose-escalation phase received a median of 2 (1-10) prior therapies with 28 having received prior bortezomib and 15 refractory to last bortezomib based therapy (12 progressing under or within 60 days of this therapy). Responses of a partial response (PR) were observed in 36/47 (76%) dose-escalation patients and 10/15 (66%) patients with bortezomib-refractory MM (Table 1). The partial response (PR) rate was 64% (30/47) and 40% (6/15) among patients with bortezomib-refractory MM. Response assessments are also available at this early cut-off date, for 12/15 patients in the dose-expansion phase with ≥PR observed in 9/12 patients (75%) including 1/4 bortezomib-refractory patients. Safety data was available for all 62 patients and common grade 3/4 adverse events included thrombocytopenia (n = 47), neutropenia (n = 33), asthenia (n = 15), and anemia (n = 10), with no treatment-related mortality. Summary/Conclusions. The combination of panobinostat and bortezomib has a predictable and manageable safety profile with promising activity in advanced MM including in patients with bortezomib-refractory MM. Updated data from the dose-expansion phase will be presented.
NF-E2 OVER-EXPRESSION ALTERS ERYTHROCYTE MEMBRANE STRUCTURE LEADING TO PREMATURE DESTRUCTION IN A TRANSGENIC MOUSE MODEL

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Background. We have previously demonstrated that the transcription factor nuclear factor erythroid-2 (NF-E2) is overexpressed in MPN patients, irrespective of the presence of the JAK2V617F mutation. In order to investigate the role of NF-E2 overexpression in the pathophysiology of MPN, we have engineered a transgenic mouse model overexpressing NF-E2 specifically in haematopoietic cells. While this model recapitulates many features of MPN including thrombocytosis, leucocytosis, characteristic BM morphology, and transformation to acute leukaemia, surprisingly the mice do not display polycythaemia. However, spleens of NF-E2 tg mice show a large increase in iron containing histiocytes, indicating increased red cell destruction. NF-E2 has previously been implicated in the transcriptional regulation of several erythrocyte membrane structural genes, as well as in globin gene expression. We therefore tested the hypothesis that NF-E2 overexpression leads to an alteration in erythrocyte membrane structure and physiology, promoting destruction of RBCs in our tg mouse model. Aim. To elucidate the effect of NF-E2 overexpression on the RBC phenotype in a novel mouse model of MPN. Methods. Two independent transgenic mouse lines were generated which overexpress human NF-E2 under the control of the hematopoietic specific vav-promoter. Expression of RBC structural genes as well as of α and β globin was quantified by qRT-PCR from mouse bone marrow of NF-E2 tg mice and wt littermates. Osmotic fragility assays were performed as previously described. Results. NF-E2 tg mice showed a significantly increased expression of several structural erythrocyte membrane components: Erythroid Protein band 4.1 (Ep4.1, p=0.002 for wt vs tg, na15), Alpha Spectrin (p=0.009) and Band3 (Slc4a1, Solute carrier family 4 anion exchanger 1, p=0.03). In contrast, four other structural genes tested (Ankyrin-1, Beta-Adducin, Erythroid Protein band 4.2 and Dematin) were not significantly different between wt and tg animals. The expression of both α and β globin was elevated in the bone marrow of transgenic animals (p=0.03 and p=0.02, respectively). Because β globin expression was increased to a greater extent than α globin, the α/β globin ratio in tg animals fell to 0.69 compared to 0.91 in wt littermates (p=0.006). In addition, the osmotic fragility of NF-E2 tg RBCs was reduced (0.45% NaCl, p=0.01 and 0.5% NaCl, p=0.02), indicating increased rigidity. Summary/Conclusions. In NF-E2 tg mice abnormally increased expression of erythrocyte structural protein genes renders erythrocytes more rigid and, by inference, less flexible. We therefore propose that these abnormal RBCs are subject to premature phagocytosis in the spleen. In addition, the disbalance between a and b globin expression in erythrocytes from NF-E2 overexpressiontg mice contributes to their early destruction. To substantiate this conclusion, m in vivo RBC turnover measurements, which are currently ongoing, will be presented.

References
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NF-E2 OVER-EXPRESSION ALTERS ERYTHROCYTE MEMBRANE STRUCTURE LEADING TO PREMATURE DESTRUCTION IN A TRANSGENIC MOUSE MODEL
effective anti-tumor immune response and high frequencies of regulatory T cells (Treg) in peripheral blood have been reported in both hematological and solid cancers. Foxp3 is the most specific marker of a regulatory phenotype, but cytokine-producing non-suppressive effector T cells (Teff) can transiently express Foxp3 upon activation. Aims. We have analyzed the frequency, phenotype and function of circulating CD4+CD25+Foxp3+ T cells in patients with chronic myeloproliferative neoplasms (CMPNs) treated with IFN-alpha2, hydroxyurea or untreated patients. Methods. 48 patients (19 women, 29 men; median age 61, range 45-83 years) with a diagnosis of PV (n=11), ET (n=15) or PMF(n=4) according to the World Health Organisation (WHO) classification were included in this study. 18 patients were untreated, 11 patients treated with hydroxyurea and 19 patients were treated with pcyelated IFN-alpha2 long term (>1 year; mean 40 months). Nine healthy subjects were used as controls (6 women, 3 men; median age 68 years). Peripheral blood mononuclear cells were isolated and frozen using standard procedures. Flowcytometric analysis was performed after surface and intracellular staining with the following antibodies: APC-Cy7 anti-CD5, PerCP anti-CD4 FITC anti-CD127, APC anti-CD25, PE-Cy7 anti-CD45RA and PE anti-Foxp3. Results. We have analyzed the frequency of circulating CD4+Foxp3+ T cells in the CD4+lymphoid compartment of patients with CMPNs. Surprisingly, we found that patients on long-term IFN-alpha treatment had a marked increase in circulating CD4+CD25+Foxp3+ T cells (12,98%;CI 10,78-15,18%) when compared to healthy subjects (6,06%;CI 4,93-7,19%), untreated patients (6,57%;CI 5,34-7,57%) or patients treated with hydroxyurea (5,82%;CI 4,29-7,57%), P<0,001. Results are given as mean with 95% confidence intervals. When distinguishing phenotypically between activated Tregs and Teff using CD45RA and Foxp3 expression, we found a significant expansion of both subpopulations in patients treated with IFN-alpha compared to other patient categories or healthy subjects (Tregs: P<0,0001; Teff: P=0,001). Summary. To date, IFN-alpha is the only therapy which is able to induce minimal residual disease with low-burden JAK2 V617F. We believe that immunological mechanisms are key factors in eradicating JAK2-mutated cells during IFN-alpha treatment. For the first time, we have shown a marked CD4+ T cell response in these patients, and further studies are ongoing to elucidate immunological responses triggered by IFN-alpha. In perspective, we hope to bring more focus on the potential benefit of immunotherapy as frontline treatment in JAK2-positive CMPNs.

Figure 1. Frequency of CD25+Foxp3+ cells in CMPN patients.

0318 CLONALITY AND LOSS OF HETEROZYGOSITY IN MESENCHYMAL STEM CELLS OF PATIENTS WITH CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background. Little is known about Mesenchymal Stem Cells (MSC) derived from the bone marrow (BM) of patients with chronic myeloproliferative neoplasms (MPNs). It has been shown that MSCs from MPN patients with JAK2V617F positive hematopoiesis never harbor the mutation and their immunophenotype is similar to that of MSCs derived from healthy donors (HD). Previous data from our group, obtained by array-comparative genomic hybridization, have documented that recurrent cytogenetic abnormalities in in vitro expanded MSCs from MPN patients are present only at late passages (P). However, the possibility that MSCs from MPN patients can be clonal has never been thoroughly investigated. Aims. We have studied clonality and loss of heterozygosity (LOH) in ex vivo expanded BM-derived MSCs of MPN patients both at early and at late P. The study was approved by the institutional review board of the IRCCS Policlinico S. Matteo Foundation, and all patients gave written informed consent. Methods. Nine patients (3 males and 6 females) were studied: 6 patients were affected from Primary Myelofibrosis (PMF), 2 from Essential Thrombocythemia (ET), 1 from Polycythemia Vera (PV); 8 healthy, age matched individuals served as controls. MSCs were isolated and expanded ex vivo, according to standard protocols from BM biopsies. DNA was extracted by commercial kit from MSCs both at early (P2-P3), intermediate (P4-P8) and late passages (P9). Clonality was assessed by the human androgen receptor assay (HUMARA) and LOH was investigated by PCR-based analysis of selected polymorphic microsatellite markers. Results. Four out of 6 female patients were heterozygous at the locus for HUMARA. Of them, 1 (a JAK2V617F positive ET) showed a skewed pattern of X-chromosome inactivation in MSCs at P2, indicating the presence of a clonal population of MSCs at the beginning of the culture. A second JAK2V617F positive ET patient had skewed X-chromosome inactivation at P12. Assessment of X-chromosome inactivation of MSCs at P3 of this patient is ongoing. The remaining 2 patients (both affected from PMF) showed a polyclonal pattern of X-chromosome inactivation. Microsatellite analysis was performed in 2 male patients (1 PV and 1 PMF, both with JAK2V617F positive hematopoiesis): 10% of MSCs showed allelic loss at P5; the proportion of cells with LOH progressively increased at P7 and P13, reaching 90% and 100%, respectively. As expected, in all cases JAK2V617F mutation was not detectable and telomerase activity was found at low levels, as in HD-MSCs. Neither skewed X-chromosome inactivation nor LOH were observed in HD-MSCs both at early and at late P. Conclusion. Taken together, our data indicate that a proportion of MSCs with genetic abnormalities is detectable in in vitro culture already at early passages, suggesting that small clones of mutated MSCs can expand in the bone marrow of MPN patients. Further studies are warranted to assess whether these MSCs can contribute to the pathogenesis of MPNs. (486/500).

0319 APOTOMIRS EXPRESSION PROFILE IN POLYCYTHEMIA VERA PATIENTS

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Background. Polycythemia vera (PV) is a clonal disorder resulting in a multipotent hematopoietic progenitor cell that causes the accumulation of morphologically normal red cells, white cells, platelets, and their progenitors in the absence of a definable stimulus. Until now many aspects of physiopathology remain unclear and no effective treatment for curing or prevention of disease progress exists. Deregulation of apoptotic machinery might have a role in PV physiopathology. Entirely understanding of apoptotic machinery and its regulation by potential modulation of the microRNA in PV patients cells might unveil novel targets which can be translated into novel therapies. Aims. To quantify the miR-26a, miR21, miR29c, miR-130b and let-7d, expression in Polycythemia Vera (PV) patients and to correlate the data with the expression of target genes. Subjects and Methods. 13 PV patients (6 males and 7 females with a mean age of 58,46y) and 15 healthy subjects (6 males and 7 females, m=57.6y). Peripheral leukocytes were obtained by Haes-Steril method, total RNA was extracted according to Trizol® method and High Capacity® Kit was used to synthesize cDNA. Quantification of apotomirs and its target genes was performed by real time PCR and results were given as $2^{-ΔΔCt}$. Statistical
analyses were performed by Mann-Whitney tests. Results. miR26a, miR21, miR29c, miR130b and let-7d levels increased in PV (median=4.01; 7.96; 2.13; 2.52; 8.47, respectively) compared to controls (median=1.33; 1.19; 1.29; 1.05; 1.64) (p=0.005; p=0.0001; p=0.017; p=0.0177 and p=0.0011, respectively). In addition, we detected the deregulated expression of let-7f (m=2.80), bcl-2 (m=0.10) anti-apoptotic and bax pro-apoptotic (m=0.28) when compared to controls (m=0.5; m=1.52 and m=1.42, respectively) (p=0.0076; p=0.002 and p=0.0052, respectively). These data suggest that bcl-2 expression may be regulated by the miR 21. Conclusions. The results indicate that peripheral blood cells patients with polycythemia vera have some microRNA signatures and genes expression distinct from those reported in literature and suggests the potential new targets and pathways that might be modulating apoptosis process and acting in physiopathology of PV. Supported by: FAPESP 06/50094-8 and 2010/01756-3.

CD133+/JAK2V617F+ CELLS DERIVED FROM PERIPHERAL BLOOD OF PRIMARY MYELOFIBROSIS PATIENTS DIFFERENTIATE INTO HEMATOPOIETIC AND ENDOTHELIAL PROGENITORS IN CULTURE

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Background. The JAK2V617F mutation, occurring in variable frequency in 50% of Primary Myelofibrosis (PMF) patients, has been used as a prognostic marker for several years. Even though JAK2V617F has been detected in terminally differentiated or CD34+ circulating cells in PMF patient peripheral blood, its contribution to the outgrowth of the malignant clone has not been elucidated yet. Characterization of JAK2V617F+ circulating primitive stem cells may shed light on our understanding of PMF pathophysiology. Aims. To identify the prime stem cell compartment responsible for PMF development, by investigating the clonogenic potential of CD133+ cells populations derived from peripheral blood of PMF patients and by assessing their JAK2V617F mutation burden variability. Method. CD133+ peripheral blood circulating cells were isolated from JAK2V617F+ PMF patients and cultured in liquid media supplemented to induce endothelial and hematopoietic differentiation. CD133+ expanded cells were also assayed for clonogenic potential in semisolid media enriched with various growth factors. Outgrown colonies were morphologically characterized, isolated and tested for JAK2V617F allele burden by real time PCR. Results. Purified CD133+/CD45+ stem cells derived from PMF patient peripheral blood were positive for the JAK2V617F mutation and expanded in suspension cultures supplemented with differentiation-inducing growth factors. Differentiation induced cultures of isolated CD133+ exhibited two different phenotypes. CD133+ cells expanded under presence of VEGF or G-CSF were found positive when immunostained for endothelial or hematopoietic lineage markers, respectively. RT-PCR analysis for JAK2V617F allele burden in isolated colonies outgrown from individual CD133+ cells indicated variability within progeny that is, differentiated into endothelial or diverge hematopoietic lineages. JAK2V617F+ allele burden was similar in expression. CD133+ hematopoietic and endothelial colonies indicated heterozygotic mutational status to occur mostly in the endothelial and macrophage colonies, while homozygotic in more committed hematopoietic progenitors. Conclusions. Our results showed that RUNX1 activity was a determinant factor for the progression of CMML to sAML; lower transactivation activity of RUNX1 mutants was associated with a higher risk and rapid progression to sAML. Supported by grants MMH-E-99009, NHRI-EX99-7571SY and DOH00-ID-2-C-111-006.

IMPLICATION OF MPL/JAK2 PROTEIN EXPRESSION DEREGLULATION IN ABNORMAL CELLULAR PROLIFERATION OF MYELOPROLIFERATIVE NEOPLASMS

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Background. Megakaryopoiesis is a multiple stage differentiation process under the control of thrombopoietin (TPO). Megakaryocytic precursors proliferate, switch to polyploidization and stop DNA replication before terminal differentiation leading to platelet shedding. We reported that UT7 cells genetically modified to over-express the TPO receptor MPL (UT7-MPL) respond to TPO by inducing senescence and that a similar process occurs in normal megakaryocytes. In contrast, megakaryocytes from primary myelofibrosis (PF) patients lack these senescence markers, suggesting that an escape from senescence-like signaling pathways could participate in the abnormal megakaryocytic proliferation observed in myeloproliferative neoplasms (MPNs). Aims. The aim of our work was to study the involvement of MPL...
and/or the associated protein kinase JAK2 expression deregulation in this escape. **Materials and Methods.** We selected 5 UT7-MPL cell clones for their ability to escape from TPO-induced cellular senescence (these clones proliferate in presence of TPO). To study megakaryopoiesis in vitro, CD34+ cells were cultured in serum-free medium supplemented with TPO. Platelets were isolated from normal, essential thrombocythemia (ET) and polycythemia vera (PV) patients. The expression levels of MPL and JAK2 were examined in the different cell lines, primary CD34+-cells and platelets by TaqMan and Western Blot, respectively. Cell signaling was studied by Western Blot. **Results and Discussion.** TPO-induced signaling was stronger in UT7-MPL cells compared to the 5 derived clones, as determined by Western blotting for the phosphorylated forms of various key TPO/MPL downstream molecules. This correlated with MPL and/or JAK2 expression, decreased in the different clones. Accordingly, we over-expressed either MPL or JAK2 in these clones, and recovered the TPO-induced proliferation arrest and expression of the senescence markers. Thus, cellular response to TPO depends on the MPL/JAK2 protein expression levels. A weak signaling is proliferative and a strong signaling induces a growth arrest and cellular senescence. Moreover, we observed a progressive and continuous increase of MPL and JAK2 protein expressions during normal megakaryopoiesis. We then hypothesized that megakaryopoiesis could be regulated by MPL and JAK2 expression levels, allowing the transition from a weak TPO-induced proliferative signal in immature cells expressing few MPL/JAK2 to a strong signaling (due to a high MPL/JAK2 expression in more engaged cells) inducing a proliferation arrest and megakaryocytic maturation. Interestingly, MPL and JAK2 expressions were lower in platelets from ET and PMF patients compared to normal donors confirming previous data on MPL level in MPN. Based on these results, we propose that the decrease in MPL and/or JAK2 protein expression may be involved in MPN abnormal megakaryocytic proliferation, by extending the proliferative signal in immature cells, resulting in the amplification of the progenitor cell compartment. This protein expression decrease could be a shared consequence between different events leading to a myeloproliferation. **Conclusion.** We show that MPL and JAK2 protein expressions are decreased in MPN megakaryocytic cells and hypothesize that this down-regulation could be involved in the abnormal cellular proliferation characterizing MPNs.

**0324 JAK2 46/1 HAPLOTYPE PREDISPOSES TO SPLANCHNIC VEIN THROMBOSIS-ASSOCIATED BCR-ABL NEGATIVE CLASSIC MYELOPROLIFERATIVE NEOPLASMS**

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**Background.** The germline constitutive JAK2 haplotype, called G/C or 46/1, is a susceptibility factor for BCR-ABL negative classic myeloproliferative neoplasms (MPN). The mechanism underlying this haplotype and the acquired MPN remains unclear. Splanchnic vein thrombosis (SVT) is variably associated with MPNs, occurring either during the course of well defined MPNs, or as the event leading to the diagnosis of MPN. **Aim.** In this study we sought to clarify the potential role of 46/1 JAK2 haplotype in the etiology of SVT. The study was approved by the institutional review board of the IRCCS Policlinico S. Matteo Foundation, and all the patients with the 46/1 haplotype were written consent. **Methods.** Screening for the 46/1 haplotype was performed by assessing the tag SNP rs12548867 that is in complete linkage disequilibrium with this haplotype. This SNP consists in a T to C shift, with the C allele associated with the 46/1 haplotype. Analysis was performed both by a PCR reaction followed by restriction-enzyme digestion and using a commercially available RT-PCR SNP genotyping assay. The chi-square or Fisher’s exact test were used in the statistical analysis; P values <0.05 were considered significant. Results. One-hundred-sixty-four subjects with SVT were studied. In 56 of them (11 with a Budd-Chiari syndrome and 45 with portal vein thrombosis) a diagnosis of MPN was excluded. One-hundred-eight patients (52 ET, 29 fibrotic and 26 pre-fibrotic PMF; 21 unclassified MPNs) received a diagnosis of MPN-associated SVT. Fifty-six healthy subjects served as controls (CTRLs). Patients with SVT but no MPN had a C allele frequency not different from that of CTRLs (0.27; P=0.69), whereas the genotypic frequencies reflected a similar similarity. SVT-associated MPN patients as a whole had a C allele frequency significantly increased compared to CTRLs (0.440 vs 0.267, P=0.0023). This difference was principally due to an excess in V617F positive cases (P=0.0001), whereas the 46/1 haplotype was not over-represented in V617F-negative cases vs CTRLs (P=0.847). Accordingly, the JAK2 mutated patients had the highest frequency of CC genotype. SVT-associated ET and PMF (prefibrotic and fibrotic type) showed a significant increase of C allele frequency compared to CTRLs, whereas SVT-associated unclassifiable MPNs did not (0.381, p=0.1720). Moreover, the 46/1 haplotype was overexpressed in JAK2V617F-negative patients and JAK2V617F-positive ET and prefibrotic PMF (0.300) but not PMF-fibrotic type (0.187) or unclassifiable MPN (0.250). These differences, however, were not statistically significant, likely due to the small number of subjects considered. These latter results are in agreement with published data showing that patients with a proven JAK2V617F-negative MPN and Budd-Chiari syndrome showed increased frequency of the 46/1 haplotype. Conclusively, our observations would contribute to the hypothesis that the 46/1 haplotype confers susceptibility neither to SVT, nor to vein thrombosis. Rather, we suggest that the 46/1 haplotype confers susceptibility to JAK2 mutation positive MPNs presenting with SVT.

**0325 DIFFERENTIAL GENE EXPRESSION PROFILE RELATED TO LEUKOCYTOSIS IN JAK2 V617F POSITIVE POLYCYTHYEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA**

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**Background.** Recognized risk factors to thrombosis in chronic JAK2V617F positive myeloproliferative neoplasms (MPN) are age at diagnosis (over sixty) and previous events of thrombosis. Recently, two additional risk factors seem to play a key role in pathogenesis of thrombosis. Thrombopoiesis (white blood cells, WBC) counts, in addition to JAK2V617F point mutation status and allele burden- has been identified as a probable independent predictor of major thrombosis in both essential thrombocythemia (ET) and polycythemia vera (PV). However, whether leukocytosis should be simply considered a marker for vascular disease or whether elevated WBC levels actually contribute directly to causing such disorders is presently matter of many studies, to be corroborated by prospective studies as well as JAK2 V617F (De Stefano et al., Am J Hematol. 2010). **Aims.** We aimed to analyze the differential gene expression profile of JAK2 V617F positive PV and ET, with and without leukocytosis (threshold, WBC>11 X109/L) before and after treatment with Hydroxyurea (HU). We hoped to identify underlying molecular alterations related with leukocytes, platelets activation and/or endothelium adhesion that may contribute to thrombosis. **Methods.** Twenty-one PV (10 with treatment, 11 at diagnosis), and 28 ET (16 with treatment, 12 at diagnosis) were included in the study. CDNA of granulocytes from healthy donors and peripheral blood mononuclear cells (PBMC) were prepared both by a PCR reaction followed by restriction-enzyme digestion and using a commercially available RT-PCR SNP genotyping assay. The chi-square or Fisher’s exact test were used in the statistical analysis; P values <0.05. **Results.** Thirty genes were significantly over-expressed in ET and PV with leukocytosis at diagnosis compared to those with normal leukocytes. Among them are to be outlined genes involved in leukocytes activation, and endothelium adhesion - CD44 (P=0.016) and SELL (P=0.009) - other genes playing key roles as transcription factors - LYN (P=0.04) -, as tyrosine kinases - JUN (P=0.013), IGF1R (P=0.004), - as tyrosine kinases in MAP kinase (MAPK) signaling pathways - MAPK1 (P=0.031), MAPK4 (P=0.009) -, in proliferative pathways - JUN (P=0.013), IGF1R (P=0.004), - as tyrosine kinases - BTK (P=0.007) -, and in regulation of hematopoietic cell differentiation and development of lymphocytes - IKZF1(P=0.000). Interestingly, over-expression of these genes disappeared after treatment with HU. **Conclusion.** Neutrophil activation correlated with activation of both endothelial cells and the coagulation cascade is a well known phenomenon in ET and PV as well as that white cells contribute to the procoagulant response at sites of vascular injury (Bouchard et al. & Curr Opin Hematol,2001; Falanga et al., Blood 2000). Our results suggest an association between leukocytosis and a group of genes involved in activation of leukocytes, and endothelium adhesion, that could contribute to the underlying molecular mechanism to thrombosis in ET and PV. In addition, genes playing key role as transcription factors and in proliferative pathways are over-expressed in PV and ET with leukocytosis at diagnosis, that may participate to thrombosis as well. In addition, disappearance...
PRV-1 OVEREXPRESsION IN ESSENTIAL THROMBOCYTHEMIA CORRELATES WITH TRAIL EXPRESSION
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Background. PRV-1, a molecular marker for Myeloproliferative Neoplasms (MPN), is a cell surface receptor expressed in blood cells, which is involved in cell signaling proliferation process. Essential Thrombocytemia (ET) and Primary Myelofibrosis (PMF) are MPN characterized by accumulation of myeloid cells in peripheral blood and bone marrow hyperplasia. Apoptosis process deregulation may be linked to the cell accumulation and, therefore, to the MPN physiopathology. Aims: 1) To determine PRV-1 gene expression in CD34+ cells and leukocytes from ET and PMF patients; 2) To correlate these results with extrinsic apoptosis related gene expression (c-flip, fas, fasL, bax, bcl-2, bcl-xL and TRAIL expression and with JAK2V617F mutation allele burden. Subjects and Methods. This study analyzed CD34+ cells and leukocytes from 22 ET patients (5 males and 17 females; mean age (ma)=58.4 years), 12 PMF patients (9 males and 3 females; ma=61.6y) and 44 controls (20 males and 24 females; ma of 46.5 y) as control group. The JAK2V617F allele burden was determined by real time allelic discrimination PCR assay. Ficoll-Hypaque protocol and Milteny CD34 isolation kit were used to obtain bone marrow CD34+ HSC whereas leukocytes were obtained by Haes-Steril method. Total RNA was extracted and cDNA synthesized by reverse transcription using High Capacity Kit. Gene expression was quantified by real time PCR as fold change (2^(-ΔΔCt)) and statistical analyses were performed by Mann-Whitney and Spearman tests. Results: There was no significant difference in PRV-1 expression in CD34+ cells from control, ET and PMF groups (p>0.05). However, PRV-1 expression was increased in ET and PMF leukocytes (median=1.97 and 4.40, respectively) compared to control (median=0.91) (p=0.014; p=0.002, respectively). Regarding JAK2V617F mutation, PRV-1 expression in leukocytes from ET and PMF patients and JAK2V617F allele burden showed a positive correlation (r=0.420, p=0.026; r=0.537, p=0.047, respectively). Considering that TRAIL expression was decreased in ET and PMF leukocytes (r=-0.28, p=0.49) in comparison to control (r=-1.6, p=0.0004, p=0.029), it was interesting to emphasize that we found a negative correlation between PRV-1 and TRAIL expression in ET leukocytes (r=-0.6228, p=0.001). Conclusions. PRV-1 overexpression is associated with apoptosis related gene expression. TRAIL, a relevant molecule in death receptor pathway and a potential therapeutic target in neoplasms. These results indicate that TRAIL deregulation and PRV-1 overexpression may contribute to the myelocaccumulation and to the physiopathology of the Myeloproliferative Neoplasms.

Expression of Leukemia Inhibitory Factor (LIF) is Increased in Myeloproliferative Neoplasms
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Background. The JAK2/STAT5 signalling pathway transduces signals from many cytokines and growth factors, and promotes essential hematopoietic events such as proliferation, cell migration and apoptosis. This pathway is frequently altered in chronic myeloproliferative neoplasms (MPNs) with activating mutations in JAK2. However, one JAK2 mutation (V617F) leads to the development of distinct phenotypic features. Some explanations have been proposed to account for this fact (such as V617F allele burden, other mutations in TET2 and other genes), but none of them fully explains the phenotypic differences observed in this group of diseases. This suggests that there might be other genes transcriptionally regulated by STAT5 that could contribute to the phenotypic variability of chronic MPNs. Methods. We performed a genome-wide search for the presence of STAT5-binding sites in human promoters. Affymetrix Exon 1.0 Arrays (Affymetrix Inc, Santa Clara, CA) were used to search for genes differentially expressed after activation of the JAK2/STAT5 pathway in cell lines. We validated expression changes by qRT-PCR. Cell lines used: - M07e, a myeloid cell line in which the JAK2/STAT5 pathway can be induced by IL-3 and inhibited by a specific inhibitor (STAT5 inhibitor, Cat. No. 573108, Calbiochem, San Diego, CA, USA). - HEL, a human erythroleukemia cell line that harbors the V617F activating mutation. − SET2, a cell line derived from a patient with essential thrombocytemia at megakaryoblastic transformation, heterozygous for the V617F mutation in JAK2. In HEL and SET2 cells, JAK2/STAT5 pathway was inhibited by the JAK2 inhibitor AG490 (from the tyrphostin family of tyrosine kinase inhibitors, Cat. No. 658401 Calbiochem, San Diego, CA, USA). To confirm whether STAT5 binds to putative binding sites in the promoter of specific genes, we measured binding by chromatin immunoprecipitation (ChIP) with a specific STAT5 antibody and qPCR, so as to compare the amount of DNA bound by STAT5 before and after IL3-mediated induction of the pathway in M07e cells. We obtained peripheral blood smears from patients with chronic MPNs and measured the expression of LIF and OSM by qRT-PCR. Results: Bioinformatic analyses predicted STAT5-binding sites in the promoter of Leukemia Inhibitory Factor (LIF). ChIP experiments confirmed binding of STAT5 to one of these motifs in response to IL-3 mediated activation of the pathway.

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Moreover, LIF expression was significantly upregulated by IL-3 in CD34+ cells and this was reversed by treatment with a specific STAT5 inhibitor. Treatment of HEL and SET-2 cell lines with a JAK2 inhibitor downregulated LIF expression. These results confirm that LIF behaves as a direct transcriptional target of STAT5. Finally, MPN patients showed significantly increased expression levels of LIF as compared with healthy donors. The finding of LIF as a novel STAT5-regulated gene might help to understand STAT5-mediated oncosgenesis.

**0329**

**LOW EXPRESSION OF GALECTIN-1 IS CORRELATED TO DOWN-REGULATION OF BAX PRO-APOTOTIC GENE EXPRESSION IN CD34+ HEMATOPOIETIC STEM CELLS FROM POLYCYTHEMIA VERA PATIENTS**


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Background. Polycythemia vera (PV) is a clonal hematopoietic stem cell malignancy characterized by an accumulation of mature myeloid cells in bone marrow and peripheral blood. A specific point mutation in the Janus kinase 2 gene (JAK2V617F) has been identified in more than 90% of patients with PV however many aspects of pathogenesis and the description of diagnostic markers remains unclear. Apoptosis deregulation may play a role in PV physiopathology and we speculate that galectin-1 interferes in the apoptotic pathway of PV patients' myeloid cells. Galectin-1 may interact with proteins from Bcl-2 family, leading to apoptosis. The potential regulation of apoptotic machinery by this lectin in PV patients cells might emerge as novel target for therapy manipulation or as a possible diagnostic marker for PV. Aims. 1) To quantify galecitin-1 and genes from Bcl-2 family expression in CD34+ hematopoietic stem cells in PV patients and controls; 2) To correlate the results of galecitin-1 expression and apoptosis-related genes and JAK2V617F allele burden. Methods. Bone marrow CD34+ cells from 20 PV patients (9 males and 11 females with a mean age of 62,25y) and 15 healthy subjects (9 males and 6 females, m=29,73y). CD34+ cells were enriched by using the MACS (magnetically activated cell sorting) CD34 isolation kit (Miltenyi Biotech). Total RNA was extracted according to Trizol method and the High Capacity Kit was used to synthesize cDNA. The expression of galecitin-1 and apoptosis-related genes was performed by real-time PCR. The JAK2V617F mutation and the allele burden were conducted by real time allic discrimination PCR. The gene expression results were given as 2-ΔΔCt. Statistical analyses were carried out by Mann-Whitney and Spearman tests. Results. Galectin-1 mRNA levels in CD34+ bone marrow cells were decreased in PV patients (median=0.68) in comparsion to controls (m=1.01, p=0.017). Pro-apoptotic bax (m=0.09) and the anti-apoptotic mcl-1 (m=3.74) expression were different from controls (m=1.049, m=1.415; respectively) (p=0.027, p=0.027; respectively). Galectin-1 expression correlated with the expression of pro-apoptotic gene bax (r=0.46; p=0.032). The bax expression also correlated with JAK2V617F mutation allele burden (r=0.46; p=0.068). Conclusions. Apoptosis impairment in myeloid cells from PV patients may be linked to low levels of galecitin-1 and bax pro-apoptotic mRNA and to the overexpression of mcl-1 anti-apoptotic gene. These findings highlight the potential participation of galecitin-1 in the physiopathology of PV, suggesting an interaction of this lectin with Bcl-2 family members in PV. Supported by FAPESP: 06/50054-8 and CAPES.

**0330**

**NO EVIDENCE THAT MPL, NPM1 OR FLT3 CONSTITUTIONAL HAPLOYPES PREDICT THE ACQUISITION OF ACQUIRED MUTATIONS IN MYELOID DISORDERS**

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Background. We and others have previously shown that the constitutional JAK2 46/1 haploypes predisposes to the acquisition of JAK2 V617F in myeloproliferative neoplasms, and to a lesser extent it also predisposes to the acquisition of JAK2 exon 12 and MPL mutations. It is not known whether this association between germline and somatically acquired factors reflects something unique about the JAK2 locus, or whether other acquired driver mutations in cancer also arise on specific inherited haplotypes. Aims. To investigate the relationship between acquired mutations and haplotypes in MPL, FLT3 and NPM1 in MPN&MPMR-EuroNet. Methods. For each gene regions of low genetic recombination were determined firstly by visual inspection of HapMap data and then minimal regions of high linkage disequilibrium were selected for detailed analysis using the programs LDMapper and PHASE along with SNP data generated by the Welcome Trust Case Control Consortium (WTCCC) from normal blood donors. From the list of haplotypes generated, a minimal number of SNPs for each gene locus was identified that could capture at least 85% of the genetic variation in that region. Pyrosequencing, a technique that provides a quantitative readout of SNP allele ratios, was used for genotyping and also detected allele skewing of heterozygous SNPs bought about by acquired uniparental disomy at 1p (MPL) or 13q (FLT3). For cases carrying homozygous MPL W515X and FLT3-ITD mutations, haplotypes associated with mutant alleles could be determined. Allele frequencies of patient subgroups were compared to data from either the WTCCC UK blood donor cohort (n=1500), the ‘German Kooperativer Gesundheitsforschung in der Augsburg’ cohort (n=1500), and the Italian ‘Invecchiare in Chia- nese’ and ‘Invecchiare in Chiangi’ cohort (n=1200) depending on the ethnicity of the patient population. The allele frequencies of each patient subgroup were compared to controls using Fisher’s exact test (2-tailed). Results. There were no significant deviations from the expected allele frequencies of matched control populations for three MPL SNPs genotyped in 144 AML cases with a diagnosis of AML and confirmed positive MPL W515X. Likewise, for the 96 cases with a diagnosis of AML carrying a mutation in MPL, there were no significant differences in allele frequency for two SNPs in MPL. For FLT3, a total of three SNPs were genotyped in 144 AML cases (FLT3-ITD; n=91, FLT3-TKD; n=43, FLT3-ITD and TKD; n=10), but again there were no statistically significant deviations from allele frequencies found in matched controls. Summary. These findings show myeloid-specific mutations in MPL, FLT3 and NPM1 appear to arise randomly on different haplotypes. Whilst this does not preclude the possibility that somatic mutations in other genes might occur preferentially on particular haplotypes, it does suggest that the association between JAK2 46/1 and somatically acquired mutations in JAK2 and MPL is not a general phenomenon.

**0331**

**FIRST YEAR ACHIEVEMENTS OF MPN&MPNR-EURONET (COST ACTION BM0902), A NEW EUROPEAN NETWORK DEDICATED TO THE DIAGNOSIS OF MYELOPROLIFERATIVE NEOPLASMS AND HEREDITARY ERYTHROCYTOSIS AND THROMBOCYTOSIS**


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Background. The MPN&MPN-EuroNet network, created in 2009, is supported by the European program COST (CoOperation in Science and Technology). It is open to all colleagues active in the fields of myeloproliferative neoplasms (MPN) and related hereditary diseases (MPNR: hereditary erythrocytosis and thrombocytosis). Aims. To facilitate and improve the diagnosis of MPN and MPNR and to establish a network in Europe. Methods. MPN&MPN-EuroNet has formed 14 working groups (WG); WG 1 focuses on JAK2-mutated MPN; WG 2 is dedicated to thrombocytthemia and myelofibrosis without mutation of JAK2 and includes subgroups specialized in hereditary thrombocyto-
in protein expression between PV and ET samples. Three spots were especially interesting in the context of our model. These corresponded to HSPA1A, a chaperone related with GATA-1 and erythropoiesis differentiation. FCM of the granulocytes showed over-expression of CD44 in PV population versus ET (P=0.004). FCM CD44 results were confirmed by bone marrow biopsies IHC in granulocytes (P=0.059). IHC also showed MMP14 differential expression between ET, PMF or PV compared to healthy biopsies in megakaryocytes, over-expressed in MPN. However, ET and PV did not show MMP14 differential expression. Significant differences of inhibition BFU-E growth and cell proliferation were found between treatment groups (Marimastat -100µM and 50µM and anti-CD44 -10mg/ml and 1mg/ml) versus group without treatment (P=0.05 and P=0.037 respectively). FCM of BFU-E cultures pointed to a significant decrease of CD71 (erythroid) and increase of CD45 (leukocyte-common antigen) with both treatments. Conclusion: Our results suggest that MMP14 and CD44 could play a role in erythroid and myeloid differentiation. Differences between ET and PV may be caused by both molecules which may contribute to phenotypic divergence. Our results suggest that MMP14 and CD44 could be future therapeutic targets. Other molecules that could contribute to phenotypic divergence, such as chaperone HSPA1A, are under study.

**0333**

**ROLE OF METALLOPROTEASES IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA ERYTHROID DIFFERENTIATION**


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**Background.** Several hypotheses have tried to explain how a unique mutation is related to three different phenotypes in myeloproliferative neoplasms (MPN). Metalloproteases are a group of proteins involved in matrix remodelling, migration and differentiation processes. Our group has found differential expression of Matrix metalloproteinase-14 (MMP14) and CD44 in PV and ET JAK2V617F positive samples (E. Albizua, V. Garcia et al. Ann Hematol. 2011). To analyze phenotypic divergence between PV and ET by proteomic screening, and validation of previously MMP14 and CD44 gene expression results by protein expression analysis and cultures methods, with the objectives of identifying alternative routes for targeted therapy. **Methods.** Fifty-nine MPN were included in the study: 24 PV, 24 ET and 11 PMF. An additional 24 healthy donors were used as controls. Granulocytes from whole venous peripheral blood were isolated and the corresponding cytosolic protein fraction was extracted. PV and ET cytosolic proteomes were analyzed using 2D-DIGE gels followed by MALDI-TOF/TOF mass spectrometry analysis of the spots of interest. Results were analyzed with DeCyder v7.0 (GE) and Mascot software. Leukocytes were obtained and analyzed by flow cytometry (FCM) using anti-MMP14, anti-CD44 and anti-CD45 antibodies (BD). Bone marrow biopsies were selected to perform immunohistochemistry (IHC) with anti-MMP14 and anti-CD44 antibodies (R&D). Finally a culture study was performed. Mononuclear cells from patients were extracted and seeded in Methocult with IL-3, SCF and EPO (Stem Cell). MMP14 was inhibited by the drug Marimastat at 100µM, 50µM and 10µM (TOCRIS); and CD44, with anti-CD44 antibody at 10mg/ml, 1mg/ml and 0.1mg/ml. Results were analyzed by BFU-E colony forming ability study by trypan blue and FCM employing antibodies anti-CD45, anti-CD71, anti-CD44, anti-MMP14 and Annexin (BD). The Mann-Whitney non-parametrical statistical hypothesis test was used to assess the statistical significance of our results. Results: 2D-DIGE analysis found 50 spots with statistically significant differences in protein expression between PV and ET samples. Three spots were especially interesting in the context of our model. These corresponded to HSPA1A, a chaperone related with GATA-1 and erythropoiesis differentiation. FCM of the granulocytes showed over-expression of CD44 in PV population versus ET (P<0.004). FCM CD44 results were confirmed by bone marrow biopsies IHC in granulocytes (P=0.059). IHC also showed MMP14 differential expression between ET, PMF or PV compared to healthy biopsies in megakaryocytes, over-expressed in MPN. However, ET and PV did not show MMP14 differential expression. Significant differences of inhibition BFU-E growth and cell proliferation were found between treatment groups (Marimastat -100µM and 50µM and anti-CD44 -10mg/ml and 1mg/ml) versus group without treatment (P=0.05 and P=0.037 respectively). FCM of BFU-E cultures pointed to a significant decrease of CD71 (erythroid) and increase of CD45 (leukocyte-common antigen) with both treatments. Conclusion: Our results suggest that MMP14 and CD44 could play a role in erythroid and myeloid differentiation. Differences between ET and PV may be caused by both molecules which may contribute to phenotypic divergence. Our results suggest that MMP14 and CD44 could be future therapeutic targets. Other molecules that could contribute to phenotypic divergence, such as chaperone HSPA1A, are under study.

**Figure 1.** FCM dot-plot of PV patient BFU-E culture.
whose role is to extinguish cytokine signaling by inhibition of JAKs. The SOCSs KIR blocks JAK kinase-activity directly, instead, the SOCSs box motif promotes the polyubiquitination and proteosomal degradation of SOCS binding partners, like Jak2. In summary, IFN-α induces SOCS expression which inhibits TPO mediated signaling through Jak2 double inhibition. This allows IFN-α and the TPO pathway to cross-talks by means of the JAK-STAT-SOCS cascade. Aims. In order to predict IFN treatment responsiveness, we evaluated the expression of specific genes involved in the IFN-α receptor pathway, which signal cross-talks with the JAK-STAT pathway under the TPO receptor. In particular we evaluated the mRNA expression of JAK1, TYK2, STAT1, STAT3, SOCS1 and SOCS3. Methods. Among the 60 IFN-α treated patients we considered eligible the 21 who had not received previous therapy and were treated with the same schedule (IFN-α 2b 3 million units 3 times a week for at least 6 months). Informed consent for the study was obtained from all patients. Two patient groups were selected on the basis of clinical response to therapy: Responders (R) (n=12) achieved a reduction of platelet count below 400x10^9/L, whereas the No Responders (NR) (n=9) group failed to show this hematological response. Target genes mRNA expression was explored by RT-q-PCR, using SYBR Green detection, on bone marrow samples. Data were normalized as follows: [mRNA normalized copy number (NCN)= mRNA target gene/mRNA GUSB*104]. Methylation-specific PCR was performed to investigate the methylation status of the promoter regions of the SOCS3 genes. Results. STAT3 expression showed a significant lower levels in R in relation to NR (p=0.01); JAK1 expression was lower in BM cells from R compared with NR (p=0.0008). No differences were found for other gene expressions and for the methylation of SOCS3 promoter between R and NR. Response was not influenced by age (p=0.06), gender (p=0.57), baseline values of hematocrit (p=0.57), platelet (p=0.52), WBC (p=0.08), spleen volume (p=0.09), JAK2V617F mutation (p=0.33). Conclusions. JAK1 and STAT3 gene expression may be used as predictor marker of response to IFN-α in ET patients. Thus, patients with low levels of JAK1 and STAT3 mRNA could be also candidate for lower IFN-α doses, which are better tolerated. Moreover, patients with JAK1 mRNA increased levels could be addressed for JAK1/JAK2 inhibitors therapy.

**0334**

**JAK2V617F ALLELE BURDEN IS A CONTRIBUTING FACTOR IN PROTHROMBOTIC MECHANISMS ACTIVATION**

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**Introduction.** Myeloproliferative neoplasms (MPN) are associated with thrombohemorragic diathesis which, in some extent, can be consequence of platelet and leukocyte activation. Different groups suggested an association between JAK2V617F mutation and thrombosis in MPN patients. **Objective.** Evaluate haemostatic activation parameters and their relationship with JAK2V617F allele burden and thrombosis in a group of PV and ET patients. **Methods.** 28 PV and 46 ET patients (median age 65 and 75y, respectively) and a control group of 47 healthy volunteers (median age 80y) were studied. All patients are clinically stable and under HU treatment. With patients’ informed written consent, aspirin was withdrawn for 10 days prior the studies. Using flow cytometry we evaluated: platelet P-selectin (CD62P) and granulophsis (CD63), platelet dense granules (mepacrine uptake/release test), platelet-leukocyte aggregates (PLA), leukocyte CD11b and monocyte Tissue Factor (TF) expression. JAK2V617F allele was quantified by Allele Specific qRT-PCR (JAK2MutaQuant, Iqos) and identified by direct sequencing. **Results/Discussion.** 28 PV (100%) and 28 ET (61%) with JAK2V617F mutation and 1 ET with MPL W515L mutation; 7 PV and 16 ET patients had thrombosis at diagnosis. All patients have increased baseline CD62P and CD63 expressions (p<0.01), increased response to arachidonic acid (p<0.01) and diminished response to TRAP6 stimulation (p<0.01), 77% of PV and 50% of ET present a storage pool disease. Leukocyte CD11b and monocyte TF expressions were increased in all patients (p<0.01). PLA were found increased in all patients (p<0.01) vs controls, nevertheless platelet-neutrophil (PMN) aggregates were significantly increased in ET vs PV (p<0.01). Patients with JAK2V617F>50% present a significantly increase of CD11b expression and platelet-PMN aggregates comparing to JAK2V617F<50% (p<0.01). PV patients present increased levels of TF when comparing with ET (p<0.01), and was higher in PV JAK2V617F>50% (p<0.01). In ET JAK2V617F mutation was statistically correlated with thrombosis and with JAK2V617F allele >50% (p=0.03 association in PV). Regarding the allele burden and platelet function studies no association was found (p=ns). These results show, with statistically significance, that PV and ET patients have circulating activated platelets and leukocytes and increased PLA. Activation parameters were higher in patients with JAK2V617F allele burden >50% comparing with <50%, which is consistent with the influence of JAK2V617F allele burden in leukocyte activation. **Conclusions.** Our data demonstrate that JAK2V617F mutation drives mechanisms that favor thrombosis, namely, neutrophil and monocyte activation, increased monocyte TF expression and platelet-leukocyte aggregates.

**0335**

**C-CBL MUTATIONS IN V617FJAK2 POSITIVE CHRONIC MYELOPROLIFERATIVE NEOPLASMS**


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**Background.** BCR-ABL1 negative chronic myeloproliferative neoplasms (CMPNs) are a heterogeneous group of clonal hematological malignancies. The most frequent aberration is V617F/JAK2, present in more than 50% of ET and ME. In last years, different groups have described mutations in other genes as C-CBL (Casitas B-lineage Lymphoma), that encodes for an E3 ubiquitin ligase involved in negative regulation of several tyrosine-kinases, as EGFR, FGR or SYK. These mutations are present in different myeloproliferative neoplasms, especially in CML, MM, and often in patients negative for other mutations, especially in ET. Some authors have described mutations in the RING finger domain of C-CBL result in deregulation of downstream targets of this protein. **Methods.** We have used dHPLC to detect sequence mutations on samples from 377 BCR-ABL1 negative CmPN patients (145 V617F/JAK2 negatives and 232 positives) and 20 samples from healthy individuals as controls. We analyzed the proliferative response induced by the C-CBL mutants in 32D cell line, previously transfected with FLT3 in four independent experiments, using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay. **Results.** We have found five mutations in C-CBL in six patients, four of them non-described previously. Three of these mutations were located in the RING finger domain, but two were present in the proline-rich domain (one of them was recurrent). All the mutations promoted a significant increase in the rate of proliferation induced by cytokines. Three of the mutations (two of the RING finger and one in proline-rich region) were found in four V617F/JAK2 positive patients. **Conclusion.** Our results suggest that mutations in C-CBL and JAK2 genes are not exclusive events. In addition, proline-rich region mutations can induce the same proliferative effect than RING finger domain mutants, so this region of C-CBL must also be considered for mutation analysis of this gene in CMPN. This work has been funded with the help of the Spanish Ministry of Science and Innovation (SAF 2007-62473), the PIUNA Program of the University of Navarra and the Caja Navarra Foundation through the Program “You choose, you decide” (Project 10830). PA has a predoctoral grant from the Government of Navarra.

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Figure 1. JAK1 and STAT3 mRNA expression is lower in IFN-α r.
O336 INCREASED PHOSPHO-MTOR EXPRESSION IN AN EX VIVO MEGAKARYOCYTIC UNILINEAGE SYSTEM DERIVED FROM CD34-POSITIVE CELLS ISOLATED FROM PATIENTS WITH ESSENTIAL THROMBOCYTHEMIA AND MYELOFIBROSIS

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Background. The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that functions as a key regulator of cell growth, protein translation and metabolism. Consistent with its role as a growth factor, numerous studies have found increased mTOR signaling in a broad spectrum of human cancers. Essential thrombocythemia (ET) and myelofibrosis (MF) are BCR-ABL-negative chronic myeloproliferative disorders characterized by megakaryocytic bone marrow hyperplasia and a sustained elevation of platelet number with a tendency for thrombosis and hemorrhage. However, the molecular mechanisms underlying the pathogenesis of these diseases are still poorly understood, delaying the development of effective targeted treatments. Aims. To evaluate phospho-mTOR expression in megakaryocytic cultures derived from the peripheral blood of healthy individuals or from patients diagnosed with ET or MF. Methods. We have developed a liquid megakaryocytic (MK) unilineage system that reproduces, ex vivo, all the stages of megakaryopoiesis generating morphologically and functionally mature platelets. Human CD34-positive cells were purified by positive selection. Unilineage MK differentiation was induced in each sample (healthy individuals and ET or MF patients) by culturing CD34-positive cells for 14 days in the presence of thrombopoietin. Purity of the selected population was evaluated immediately after purification and during MK differentiation (days 0, 3, 6 and 12) by flow-cytometry using anti-CD34 and anti-CD61 antibodies and morphological analysis after May-Grünwald-Giemsa staining. Phospho-mTOR expression was analyzed on day 3 and 12 of the differentiation process by flow-cytometry, immunofluorescence and immunohistochemistry (carried out on slides from 35 bone marrow samples: 14 ET and 21 MF patients). Results. mTOR activation was increased during MK differentiation in both ET and MF patients compared to healthy donors where mTOR staining was barely detectable. Immunohistochemical analysis of phospho-mTOR confirmed high expression levels in ET and MF patients in contrast with the negative staining observed in healthy individuals. Conclusions. Taken together, our data suggest that induction of the mTOR pathway is involved in the MK differentiation of samples derived from ET and MF patients. Our findings suggest that mTOR could represent an attractive molecular target for the treatment of ET and MF patients failing previous lines of treatment.

O337 MOLECULAR PROFILING OF PERIPHERAL BLOOD CELLS FROM PATIENTS WITH POLYCYTHEMIA VERA AND RELATED NEOPLASMS IDENTIFIES SIGNIFICANT Deregulation of inflammation GENES

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Background. Essential thrombocythemia (ET), polycythemia vera (PV) and primary myelofibrosis (PMF) are haematopoietic stem cell neoplasms, in which the JAK2 V617F mutation is observed in >95% of patients and in about 30% of PMF patients. All diseases may be associated with autoimmune or chronic inflammatory disorders. Gene-expression profiling studies have yielded divergent results which might be explained by different platforms used and differences in the cell types being profiled (granulocytes, CD34+ cells) and their origin (bone marrow samples, peripheral blood). aberrant expression of genes involved in inflammatory responses has also been reported, mainly being performed on granulocytes or CD34+ cells. Aims. In order to achieve a “global” characterization of aberrant genes of significance for inflammation and immune function, we have performed gene expression profiling of whole blood to obtain an integrated transcriptional signature of cells involved in inflammation, immune surveillance and function. Methods. Gene expression microarray studies have been performed on control subjects (n = 21) and patients with ET (n = 16), PV (n = 36), and PMF (n = 9). Patients on interferon-alpha therapy were excluded from the study. Gene expression profiles were generated using Affymetrix HG-U133 2.0 Plus microarrays recognizing 54,675 probe sets (58,500 genes). Total RNA was purified from whole blood and amplified to biotin-labeled RNA and hybridized to microarray chips. Results. We identified 23,657, 25,567, and 17,417 probe sets which were significantly differentially expressed between controls and patients with ET, PV, and PMF, respectively. The most significantly upregulated genes included in ET patients CCL7, IL8, FGB, CCR5, IL1R1, FGL1, IL1B, CCR3, CCL25, FGB, and ITGB3, in PV patients CCR1, CXCL10, IL1R1, and ORM1, and in PMF patients C5, CXC12 CXC13, ORM1, PTX3, and VEGFA. In the whole group of patients, 87 genes were significantly downregulated. The C5 and I5L genes were progressively and significantly upregulated in patients with ET, PV, and PMF, whereas the genes, CCR3, CCR6, CCR9, CD40LG IL10RA, and SELPLG were progressively and significantly downregulated from ET over PV to PMF, respectively. Summary/Conclusions. Our findings of significantly deregulated genes involved in inflammation and immunoregulation with progressive deregulation for particular genes from ET, over PV to PMF may reflect chronic inflammation to be of pathogenetic importance for the progression of these neoplasms towards the myelofibrotic end-stage. In this context, the aberrant inflammatory and immunoregulatory pathways may be deregulated as part of the clonal evolution. Irrespective of the underlying mechanisms, the deregulated (upregulated or downregulated) genes may drive chronic myeloproliferation. It is intriguing to consider if chronic inflammation or a chronic aberrant autoimmune process might also elicit clonal evolution and the emergence of a myeloproliferative neoplasm. If so, the abnormal immune homeostasis may be involved in the progression of the disease consequent to defective immune surveillance.

O338 THE ACTIVATING G537R MUTATION IN HIF2-ALPHA IS ASSOCIATED WITH IN VITRO HYPERSENSITIVITY OF ERYTHROID PROGENITORS TO ERYTHROPoietin

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Background. Polycythaemia/erythrocytoses in childhood are rare and may be a result of various pathophysiological conditions. Primary polycythaemias were reported in children with acquired mutations in JAK2 and with congenital mutations in erythropoietin receptor (EPOR). recessive mutations in gene encoding von Hippel-Lindau (VHL) protein lead to polycythaemia, which exhibits features of both primary and secondary. Recently described polycythaemic patients with mutations in PHD2 and HIF2-alpha (HIF2A), were reported to have elevated serum EPO levels; the information on in vitro sensitivity of erythroid progenitors to EPO is missing or not complete. Aim. We aim to characterize, at cellular and molecular level, selected pediatric patients diagnosed with polycythaemia in Czech Republic (1990 - 2010). Part of this cohort (6 patients) was previously presented. Patients and Methods. We studied a group of 9 children diagnosed at the centers of Pediatric Hemato-Oncology in Czech Republic with signs of primary polycythaemia. The diagnosis were in the range of 18.2-23.2 g/dL. Four patients were clinically asymptomatic, five patients had plethora. Hematopoietic colony assay was used to determine in vitro sensitivity of erythroid progeni-
ators to EPO. Mutation analysis of known candidate genes was performed and included sequencing of JAK2 exons 12 and 14, EPOR exons 7 and 8, HIF2A and HIF1A exon 12 and all exons of VHL and PHD2. Results. All patients exhibited in vitro hypersensitivity of erythroid progenitors to EPO, seven samples were also positive for the growth of endogenous erythroid colonies (EECs). A known heterozygous 5967insT mutation in EPOR was found in two unrelated patients; this result was already presented. Recently, we detected another previously reported mutation, heterozygous 1609G>A substitution which changes glycine 537 for arginine in HIF2A, in the other two unrelated and clinically asymptomatic patients. The erythroid progenitors of both were in vitro hypersensitive to EPO, one was also EEC-positive. Surprisingly, their EPO levels were not elevated, however only one single measurement for each from two different biochemical laboratories is available. Therefore EPO levels need to be re-evaluated in one center using another rigorous measurement. The remaining five patients were negative for a mutation in all analyzed genes. Conclusions. We present a group of pediatric patients with pri-
mary polycythemia, and mild clinical sings in 5/9 of the cases. The dis-
ease causing mutation was detected in 4/9 patients. The presence of the same G537R mutation in two our patients in combination with in-
dependent cases reported by others confirm the suggested mutational hotspot in HIF2A gene. We show for the first time that in addition to VHL-associated polycythemia also erythroid progenitors of patients with HIF2A mutation are in vitro hypersensitive to EPO. We hypothe-
size that endogenous erythroid EPO production is stimulated by this activating G537R HIF2A mutation leading to augmented activation of EPO/EPOR pathway and excessive erythroid proliferation.

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0339

ANALYSIS OF THE MUTATIONAL STATUS OF IL-3, IL-5 AND GM-CSF RECEPTORS IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS

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Background. Over the last few years several genetic aberrations have been described to be the cause of some cases of chronic myeloprolif-
erative neoplasms (CMPNs). Among these aberrations V617F muta-
jion in JAK2 is the most frequent one. Having been found in 55% of ETs, almost 100% of PVs and 65% of IMFs. In spite of these findings, an important number of CMNPs still have an unknown molecular origin. Aims. Current knowledge of CMNPs has revealed the impor-
tance of JAK2 and the JAK/STAT pathway in these diseases. This en-
couraged our team to study cytokine receptors signaling through JAK/STAT pathway. We focused our work in the screening for muta-
tions on the genes coding for the receptors for interleukin (IL)-3, IL-5 and GM-CSF. Methods. We analyzed a series of DNA samples from 44 patients with BCR-ABL1 and V617FJAK2 negative CMNPs. We used denaturing High Performance Liquid Chromatography to carry out a screening for mutations on the coding exons of genes IL3RA, IL5RA, CSF2RA and CSF2RB. Samples with an elution profile different to a healthy control were sequenced. For the exons with alterations the study was extended to other series of CMNPs patients, either V617FJAK2 positive or negative, and a longer series of samples from healthy donors was analyzed for the recurrent ones. No matched sample from other tissue was initially available in any case. Results. We found nine sequence changes not previously described as mutations nor polymorphisms in dbSNP or 1000genomes.org in CSF2RB, CSF2RA and IL3RA. Five were found in CSF2RB (p.D312N, p.A328T, p.F509S, p.F513L, and p.R517C); three in CSF2RA (p.R164Q, p.P166S and p.Y167D); and one in IL3RA (p.W226X). R164Q in CSF2RA was found to be recurrent as it appeared in nine patients, but also in three healthy donors. In one case with R164QCSF2RA a buccal swab sample was found to carry the same sequence change, showing that it was germinal. In addition, D312N in CSF2RB and Y167D in CSF2RA appeared in one healthy donor each. Summary. We have found some sequence changes in the genes we studied. Three cases (R164Q and Y167D in CSF2RA, and D312N in CSF2RB) were also present on healthy samples. These changes seem to be rare variants, although they could contribute to the effect of other mutations. For the six remain-
ing cases the analysis of 20 control samples was negative. These sequence variants (1.10% (4/362) in CSF2RB, 0.55% (1/183) in CSF2RA, and 0.64% (1/156) in IL3RA) could be rare variants or singletons but could also be onco-
genic mutations. In fact, random mutagenesis experiments have shown an oncogenic effect for some CSF2RB mutations. In addition, W226X, found in IL3RA produces a truncated protein. We are cur-
rently studying the impact of the sequence changes we found on cell cultures. Anyway mutations in these genes, if existing, would be infre-
quent events in these diseases. Although considering all our data, al-
most 2% of CMNPs cases show alterations in these genes.
Non-Hodgkin lymphoma - Biology

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CD20 DIRECTS CELL POSITIONING IN SECONDARY LYMPHOID ORGANS

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Background. Human CD20 is a B cell restricted transmembrane molecule and the most successful monoclonal antibody targeted antigen, used worldwide to treat patients with B cell non-Hodgkin’s Lymphoma (B-NHL). Unfortunately, the majority of B-NHL patients develops resistance to anti-CD20 therapy, resulting in relapse of the disease. Anti-CD20 antibody resistance is poorly understood and a current focus of investigation. Antibody resistance may be related to the interaction of CD20 with tumor cells and the microenvironment. However, although the CD20 molecule was discovered 30 years ago, its exact function is still unknown. Aim. The aim of this study is to gain insight in the role of the CD20 molecule in relation to the microenvironment. Methods. To explore the function of CD20, we used a system that isolates human CD20 from other B cell surface molecules by retrovirally transferring the human CD20 gene into normal human T cells. Then these CD20-positive T cells were used in in vivo mouse models and in in vitro functionality assays. Results. Initial in vitro assays comparing peptide-specific CD20-positive T cell clones with nontransduced parental clones demonstrated no altered proliferative activity or cytokine production associated with CD20 transduction. We then injected a T cell population containing 20% CD20-positive T cells into immune deficient Rag2-/- Ly5.1+ mice (n=10) to compare the distribution of the cells into the organs with the distribution of normal human T cells (control mice n=10) and human B cells (control mice n=5). Immunohistochemical staining of the organs revealed a remarkable phenomenon in all 10 spleens of the mice that received the transduced T cells: while normal T cells were scattered throughout the spleen, the CD20-positive T cells had positioned themselves periarteriolar in the same way as the human B cells do (figure 1).

In the other organs, like the gut, liver and lungs, in contrast, the distribution of the CD20-positive T cells did not differ from the normal T cells. To confirm the hypothesis that the migration capacity of T cells is altered following expression of the CD20 molecule, we studied the influence of CD20 expression on T cells in in vitro transwell migration assays. In these assays CD20-positive T cells exhibited a 50% decreased migration capacity towards stroma cells compared to the normal T cells (p=0.0075). Summary/Conclusions. These data demonstrate that the CD20 molecule directs the positioning of cells in secondary lymphoid organs. This indicates that CD20 holds back further migration of the cells in order to take a periarteriolar position, which may be the optimal site for the physiological (T cell independent) B cell antigen recognition. These findings help to understand the role of CD20-positive cells in their microenvironment, which opens up new ways to conquer anti-CD20 antibody resistance in the treatment of B-NHL.

Figure 1.
Junctional adhesion molecule C (JAM-C) influences selectively the homing of normal and malignant B-cells to different lymphoid organs

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Background. Homing of malignant B-cells to bone marrow (BM) and secondary lymphoid organs is of critical importance in disease progression of lymphoproliferative syndromes. Junctional adhesion molecule C (JAM-C) is an adhesion molecule with restricted expression to pre-germinal centers (GC) and GC-dependent chemokine receptors. Guided by differentially expressed B-cell homeostatic and activation-dependent chemokine receptors.

Results. In the model of the step-wise development from a non-neoplastic lesion to the FL associated gastritis, we observed a de novo expression of CXCR7, CCR4, CCR5 and CXCR6 and de novo expression of CXCR9 and XCR1 in BM and spleen. Continuous expression of all B-cell homeostatic chemokine receptors was found in both lymphoma entities except for CXCR4, which was entirely lacking following transformation to MALT lymphoma. This expression, the ligand for CXCR7, was found in epithelial, endothelial and dendritic cells, macrophages, in lymphoma cells of gastric MALT lymphomas and with a significant higher number of positive lymphoma cells in BM. Comparing CXCR4 expression between gastric MALT lymphoma and nodal marginal B cell lymphoma and between gastric BM and nodal DLBL, CXCR4 mRNA transcripts were exclusively found in nodal lymphoma samples. The proliferation rate of gastric MALT lymphoma and gastric DLBLs correlated with expression of CXCR9 and CXCR7. Our results support a model of a stepwise progression of gastric MALT lymphoma from a non neoplastic entity to HP associated gastritis, to MALT lymphoma, and finally to overt DLBL, is guided by differentially expressed B-cell homeostatic and activation-dependent chemokine receptors.

Conclusion. Blocking JAM-C with anti-JAM-C antibodies reduced the homing of normal and malignant B-cells from mantle cell lymphoma and marginal zone lymphoma to BM and spleen by 50–60%. This contrasted to the homing of B-cells into BM and lymph nodes but not into spleen when the cells were incubated with anti-VLA4 antibodies prior to injection. Interestingly, combination of both antibodies resulted in inhibited homing into the three lymphoid organs. Conclusions. Our results show for the first time a functional role of JAM-C in B cell proliferation and in the homing to lymphoid organs. Targeting JAM-C could thus constitute a new therapeutic strategy, with JAM-C blocking as a treatment to prevent lymphoma cells from reaching supportive microenvironments in BM and spleen.

0344

Deep sequencing reveals a complex pattern of clonality in follicular lymphoma by analysis of the heavy chain of the immunoglobulin gene in different tumor sub-populations

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Background. We previously demonstrated the existence of a common progenitor cell (CPC) linking follicular lymphoma (FL) and transformed FL (t-FL) by analyzing the somatic mutation (SHM) of the heavy chain of the immunoglobulin gene (IgH-H). Following malignant transformation from HP to MALT lymphoma, an up-regulation of CXCR7 and CXCR9, de novo expression of CXCR3 and a loss of CXCR4 were detected. Additionally, we showed that the chemokine receptor expression profile of gastric MALT lymphomas differs substantially compared to eDLBCL with a higher expression of CCR1, CCR5, CCR7, CXCR5, CXCR4 and CXCR6 and de novo expression of CXCR9 and XCR1 in eDLBCL. Continuous expression of all B-cell homeostatic chemokine receptors was found in both lymphoma entities except for CXCR4, which was entirely lacking following transformation to MALT lymphoma. SDF-1 expression, the ligand for CXCR4 and CXCR7, was found in epithelial, endothelial and dendritic cells, macrophages, in lymphoma cells of gastric MALT lymphomas and with a significant higher number of positive lymphoma cells in BM. Comparing CXCR4 expression between gastric MALT lymphoma and nodal marginal B cell lymphoma and between gastric BM and nodal DLBL, CXCR4 mRNA transcripts were exclusively found in nodal lymphoma samples. The proliferation rate of gastric MALT lymphoma and gastric DLBLs correlated with expression of CXCR9 and CXCR7. Our results support a model of a stepwise progression of gastric MALT lymphoma from a non neoplastic entity to HP associated gastritis, to MALT lymphoma, and finally to overt DLBL, is guided by differentially expressed B-cell homeostatic and activation-dependent chemokine receptors.

Methods. The frequency of somatic mutations for the 14 SHM hotspots was calculated using the software SVDmuts. The SHM data were visualized using SHM plotter (http://www.shm-plotter.org). Our results show for the first time a functional role of JAM-C in B cell proliferation and in the homing to lymphoid organs. Targeting JAM-C could thus constitute a new therapeutic strategy, with JAM-C blocking as a treatment to prevent lymphoma cells from reaching supportive microenvironments in BM and spleen.

0345

The kinetics of systemic cellular immunosuppression in patients with poor-risk diffuse large B-cell lymphoma during treatment with ‘CHOP-R’: a prospective study from the ALLG

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Background. The immune system plays a pivotal role in the pathogenesis of lymphoma. Within the malignant lymph node, a variety of mech-
Background. Splenic marginal zone lymphoma (SMZL) is an indolent mature B-cell malignancy. However, nearly one-third of patients display a rapidly progressive disease and a dismal outcome. Risk stratification has been recently proposed based on the assessment of clinical and laboratory parameters on diagnosis. Biological prognostic factors are still lacking and their identification might prove of great value for identifying patients at high risk of unfavorable disease. In SMZL, bone marrow (BM) infiltration is almost invariably observed on diagnosis and the BM microenvironment may play an important role in the disease progression. Aims. Aim of this study was to characterize the BM microenvironment associated with SMZL infiltrates in order to identify potential influences of the stroma on the biology and natural history of this lymphoma. Methods. Routinely processed BM biopsies (BMB) of thirty-five consecutive cases of SMZL diagnosed at our Institution between 2001 and 2010 were collected. All patients had a BMB at the time of diagnosis. Besides the expression of CD20, CD3, CD5, CD45, CD43, CD23, CD11c−CD123−CD19+ B lineage cells, and tryptase+ cells (mast cells), the expression of CD4+CD127−CD25hi regulatory T-cells or CD14-HLA-DR− monocytes were measured. Time to progression (TTP) was used as clinical endpoint. TTP was defined either as an increase in size of previously documented disease greater than 25%, or as the appearance of disease at any new site or even the shift to a more aggressive histotype. Results. We found a significant correlation between the amount of infiltrating tryptase+ mast cells and TTP (p=0.05, p=0.03). A strong positive correlation was observed between CD40 stromal expression and the number of tryptase+ mast cells (p=0.0001). Multivariate analysis was performed including, along with tested immunohistochemical variables, clinical and laboratory features of the SMZL prognostic score system, such as hemoglobin, LDH, and albumin levels. Notably, Cox proportional hazard revealed that the amount of tryptase+ mast cells populating SMZL BM infiltrates, and CD40 expression in the BM stroma were significant and independent predictors of a shorter TTP (p=0.05; p=0.03) in our SMZL patients. Conclusions. Here we demonstrated that BM microenvironment-related features, namely the presence of tryptase+ mast cells and CD40 stromal expression, could have a role in determining prognosis of SMZL patients. Our preliminary results point out a possible functional link between mast cells and BM stromal cells that may impact on neoplastic clone survival and proliferation. Further investigations will address the functional significance of such interactions.
1995 described to play a part in CNS development and later as a potential tumor marker for MCL, as it is overexpressed in this subset of hematological malignancy and the SOX11 expression seems to give information on the clinical and biological behavior in MCL. Aims. We wished to establish a very sensitive and specific qPCR assay for detection and quantification of SOX11 mRNA in order to use this gene as biomarker for MCL. As SOX11 is an intronless gene the challenging task was to establish a sensitive and specific qPCR assay without the risk of contaminating gDNA. Furthermore we wished to compare the expression patterns of SOX11 and CCND1 in a cohort of MCL patients. Methods. Mononuclear cells were obtained from peripheral blood (PB) and bone marrow (BM) samples from healthy individuals and diagnostic PB and BM samples from MCL patients. The cell line Granulocytes were used as positive control for SOX11 and CCND1 expression. Total RNA was prepared and cDNA was synthesized using anchored oligo(dT) primers avoiding amplification of gDNA in the downstream qPCR. We overcame the risk of gDNA contamination by the design of a polyA specific reverse primer together with an LNA modified TaqMan probe ensuring efficient amplification and detection of SOX11 cDNA. GUS and B2M were used as control genes. Results. We find a highly significant differences in expression of SOX11 between healthy individuals and MCL patients (PB: p<0.014, BM: p=1.0004, calculated using Wilcoxon rank-sum test). Figure 1 depicts the SOX11 expression levels in healthy individuals and diagnostic MCL PB and BM evaluated as ∆Ct (∆Ct = Ct(SOX11)-average Ct control genes) with an assay sensitivity of 10-3. Ct = 40 was used as cut off between SOX11 positive and negative samples. Conclusions. A highly sensitive and specific qPCR assay for SOX11 without risk of contaminating gDNA was established enabling SOX11 to be used as biomarker for following MRD in MCL. This potent qPCR assay will be used in the ongoing longitudinal study of MRD in MCL patients in order to demonstrate a connection between treatment response and load of MRD in the heterogeneous group of MCL patients.
nutlin-3a in an effort to uncover new targets of activated p53 protein in our in vitro system. Results. Our preliminary proteomic data revealed that Hsp90 protein levels were significantly decreased following nutlin-3a treatment in SUP-M2 and DEL. This finding was confirmed using Western blot analysis and whole lysates of the same ALK+ ALCCL cell lines. Combined treatment of ALK+ ALCCL cells with nutlin-3a and 17-AAG, a potent inhibitor of Hsp90, which is already used in investigational clinical trials in patients with aggressive lymphomas, resulted in significant decrease of cell viability assessed by trypan blue exclusion assay. In addition, proliferation of viable cells assessed by MTS assay and total cell numbers was substantially reduced following nutlin-3a treatment in the ALK+ ALCCL cell lines. Analysis of these data also demonstrated synergistic effects in cell death and proliferation of cells treated with nutlin-3a and 17-AAG as compared with the results for each agent alone. Conclusions. Our preclinical findings support the rationale for combined use of targeted therapies such as nutlin-3a and 17-AAG in aggressive lymphoma types.

0350
ANALYSIS OF T-CELL SUBSETS BY FLOW CYTOMETRY IN LYMPH NODE BIOPSY IDENTIFIES PATIENTS WITH GOOD PROGNOSIS IN FOLLICULAR LYMPHOMA (FL)
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Background. Tumor microenvironment plays an important role in the outcome of the patients with FL. By gene expression and immunohistochemistry, an increase in macrophages has been associated with poor outcome, whilst an increase in T-cells is associated with better prognosis. The quantification of immunohistochemical staining is time consuming and poorly standardized. The use of flow cytometry could help in identifying the different groups of risk in FL patients. Patients and Methods. Lymph nodes at diagnosis from 73 patients (35M/38F; median age 59, range 29 to 81) with FL were processed by standard flow cytometry. The percentage of CD3, CD4, CD8, CD57, and germinal center (GC) CD4 cells (CD4+1CD8+), as well as the ratio B/T, CD4/CD8, CD4/CD23, CD8/CD23 and GC-CD4/CD4 were correlated with the main initial features and the clinical outcome of the patients. Histological grade 1 and 2 was observed in 58 patients, grade 3a in 12 and grade 3b in 1. Low-risk FLIPI was observed in 52%. 61 patients has received polychemotherapy, including rituximab in 36. After a median follow-up of 6 years, 25 patients have died, with a 5-year overall survival (OS) of 76%. Results. The mean (±SD) percentage of B-cells, CD3, CD4, CD8, and GC-CD4 was 59.7% (±15.1), 35.2% (±15.4), 26.4% (±12.3), 8.7% (±5.5), and 3.6% (±9.2), respectively. Age >60 years was associated with higher percent of CD8, CD38 cells and higher CD8/B-cell ratio. Grade 3 histology was associated with higher CD8 and CD57 cells and lower CD4/CD8 than grades 1 and 2. Response to treatment was not related to lymphocyte subpopulations. FLIPI, among other clinical variables, was able to predict OS. Patients with a CD4/CD8 ratio ≥4.4 had better OS than the remainder (5-year OS: 100% vs. 68%, respectively; p=0.01). Patients with high GC-CD4/CD8 ratio (0.19) showed a better OS than the others (5-year OS: 100% vs. 65%, respectively; p=0.02). A multivariate analysis identified GC-CD4/CD8 (p=0.03), CD4/CD8 (p=0.01) and FLIPI (p=0.04) as the most important variables to predict OS. Conclusion. Flow cytometry allows the identification of a microenvironment pattern associated with good prognosis in patients with FL.

0351
ACQUIRED IGH TRANSLocations in SPLENIC MARGINAL ZONE LYMPHOMA TARGET MISCELLANEOUS PARTNER GENES, INCLUDING MYB
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Background. Bone marrow biopsy (BMB) is mandatory for non-Hodgkin’s lymphoma’s (NHL) staging, being also useful for assessing treatment response and for re-staging in relapse. Immunophenotypical analysis by multivariate flow cytometry (MFC) has been increasingly used in hematological malignancies due to its high applicability and sensitivity at diagnosis and for detection of minimal residual disease (MRD). However, the use of MFC in the routine clinical settings for NHL staging is not yet well established. Aims. To compare the value of trephine biopsies (BMB) and multivariate flow cytometry (MFC) in the assessment of bone marrow infiltration in NHL. Methods. 494 diagnostic (n=282) and follow up (n=212) specimens, simultaneously analysed by MFC and MBC, from non Hodgkin’s lymphoma’s (NHL) patients were included in the study. Most patients (87%) had B-cell NHL: diffuse large B cell lymphoma (DLBCL) 35% (n=172), follicular lymphoma (FL) 27% (n=165), mantle cell lymphoma (MCL) 10% (n=49), marginal zone lymphoma (MZL) 9.7% (n=46), Burkitt’s lymphoma (BL) 2.2% (n=11), small lymphocytic lymphoma (SLL) 1.8% (n=9) and lymphoplasmacytic lymphoma (LPL) 1.4% (n=7); the rest of cases (12.8%) were classified as T/NK lymphomas (T/NK NHL). BMB infiltration was categorized as nodular, interstitial, mixed and diffuse. Selected panels of monoclonal antibodies were used for histology study, using four-colour direct immunofluorescence technique and according to previously well described methods, aimed to identify and characterize B and/or T cells in BM. Results. Concordant results between BMB and MFC were found in 396 samples (80%), being both techniques negative in 83% (320/396) and positive in 17% (66/396) of cases. Discordant results were found in 92 cases (20%), with a similar distribution among cases BMB+MFC- (42%; 41/98 cases) and BMB-MFC+ (58%; 57/98 cases). Considering histology, discordant results were found more frequently in T/NK NHL (33%; 21/68 cases) in which MFC
was a little more sensitive than BMB, since 62% of discrepant cases (13/21) corresponded to BMB+/MFC+ cases, whereas 38% (8/21) corresponded to BMB+/MFC− cases. In MZL, 23% (11/48) cases were discrepant, with a similar frequency of cases BMB+/MFC+ (45%) and BMB+/MFC− (55%). In FL and DLBCL, discrepancies were more uncommon, with 17% (23/135 cases) and 15% (26/172 cases) of discrepancy, respectively. In FL, BMB was slightly more sensitive than MFC (13/23 -56% being BMB+MFC+), and, by contrast, in DLBCL MFC was more sensitive (15/26 -55% being BO-MFC+). Although with low number of cases, all discrepancies in LPL (2/7) were BMB+MFC− whereas in BL (2/11) all discrepancies were BMB−MFC+. Summary/Conclusions. According with our results, we can conclude that MFC can detect a subgroup of patients with bone marrow involvement in which BMB is negative. Therefore both techniques complement each other and both should be used for the detection of bone marrow infiltration in B cell disorders.

0355 FREQUENT DELETION OF THE TUMOR SUPPRESSOR TNFAIP 3 IN SEZARY SYNDROME SAMPLES
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The genetic background of Sezary syndrome, a disseminated form of cutaneous T-cell lymphomas (CTCL), is still a matter of discussion. To unravel genetic imbalances, high density comparative genome hybridization was performed on leukemia samples derived from 12 patients. We identified bi- and monoallelic deletions of the tumor necrosis factor alpha induced protein 3 gene (TNFAIP3; A20) in a high proportion of SS patients as well as haemizygous A2O deletion in the SeAx sample. Furthermore, we demonstrate that inhibition of A2O activates the NF-κB pathway thereby increasing the proliferation of normal T lymphocytes. On the other hand, the reconstitution of A20 expression slowed down the cell cycle in SeAx cells. Recently A2O inactivation has been reported in various B-cell lymphomas. In this study we show that A2O is also a putative tumor suppressor in the T-cell malignancy - Sézary syndrome.

0354 PATIENTS WITH B-CELL NON HODGKIN LYMPHOMA SHOW INCREASED FREQUENCIES OF REGULATORY T CELLS AND CD8+ T-CELL EXPANSIONS
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Background. Although most non hodgkin lymphomas (NHL) take origin from the B-cell lineage, several studies suggest that any impairment involving the different branches of the immune system may play a role in their pathogenesis. Moreover the cross-talk among lymphoma cells and other cell types, such as for instance T-lymphocytes and antigen presenting cells, within the peritumoral microenvironment seem to deeply influence the onset and evolution of NHL. Aim. In order to explore the possible impact that the degree of activation of the T-cell immune system and the balance among different T-cell populations may have on the NHL pathogenesis, we analysed the T-cell receptor (TCR) repertoire and the distribution of different T-cell subsets -including regulatory T-cells (Treg)- in patients with NHL. Methods. Our study was based on a flow cytometric analysis performed on the peripheral blood of 15 patients (6 with indolent NHL and 9 with diffuse large B-cell lymphoma, DLBCL) and 15 age-matched controls. We first determined the frequency of CD8+, CD4+, CD8+ and CD16-56+ T-cells. Treg were then identified by considering the CD4+ cell fraction characterised by a very high (>2 log) expression of CD25 and by a very low (<2 log) expression of CD127, as well as by determining the expression of FoxP3 and CD152. TCR repertoire analysis was based on a panel of 24 beta variable (BV) family-specific antibodies. A BV expansion was defined as any impair-

Figure 1.
SI-1 (15.31 ± 2.09%, P=0.03) by anti-CD49f/α6 mAb. Incubation with anti-CD49c/α3, anti-α9β1, anti-αiβ3 and anti-CD104/β4 mAbs did not inhibit PEL cell adhesion to LN. FN binding was reduced by 68% for BC-3 (3.33 ± 1.70%, P=0.01) and 76% for BCP-1 (6.49 ± 4.62%, P=0.01) by anti-CD29/β1. BC-3 adhesion to FN was also reduced by 67% (3.49 ± 1.04%, P=0.01) by anti-CD49d/α4, whereas BCP-1 adhesion to FN was moderately although significantly reduced by anti-CD49c/α3 (24.76 ± 15.06%, P=0.03), anti-CD49d/α4 (22.25 ± 5.80%, P=0.01) and anti-CD49e/α5 (13.76 ± 6.64%, P=0.04) mAbs, with 8%, 18% and 49% inhibition, respectively. Moreover, competing RGD peptides significantly reduced BCP-1 attachment to FN, in a dose-dependent manner, suggesting a predominant involvement of αvβ3/5. Conclusions. All PEL cell lines express the LN receptor αvβ3/5 and at least one FN receptor, mostly α4β1/β4-A4. BBG-1, BC-3 and SI-1 cell attachment to LN is predominantly αvβ3/5-VLA-6-dependent. BC-3 binding to FN is mostly α4β1/VLA-4-dependent, whereas BCP-1 binding to FN is mediated by αvβ3/5/VLA-3, α4β1/VLA-4 and αvβ3/5-VLA-5, with a predominant involvement of αvβ3/VLA-5. These integrin receptors may represent interesting targets for the development of novel therapeutic strategies for patients with PEL.

**0356**

**HANS’ CLASSIFIER IN PATIENTS TREATED WITH DOSE ADJUSTED EPOCH-R (DAEPOCH-R) FOR ADVANCED DIFFUSE LARGE CELL B LYMPHOMA (DLBCL)**

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**Purpose.** Analysis of the Progression Free Survival (EFS) and overall survival (OS) of diffuse large B-cell lymphoma treated with R-EPOCH utilizing Han’s classifier. Material and Methods. We use R-EPOCH as described by Wilson et al. No radiotherapy was applied over bulky disease. We included only patients diagnosed of diffuse with a IPI score of 3 or IPI adjusted 65 higher than 2. Han’s classifier was applied as reported by Hans et al. No radiation therapy was applied over bulky disease. We included only patients diagnosed of diffuse large B-cell lymphoma with an analysis of germinal center and post-germinal center biomarkers. J Clin Oncol 2008;26:2717-24.

**Results.** OsI-1 (15.31 ± 2.09%, P=0.03) by anti-CD49f/α6 mAb. Incubation with anti-CD49c/α3, anti-α9β1, anti-αiβ3 and anti-CD104/β4 mAbs did not inhibit PEL cell adhesion to LN. FN binding was reduced by 68% for BC-3 (3.33 ± 1.70%, P=0.01) and 76% for BCP-1 (6.49 ± 4.62%, P=0.01) by anti-CD29/β1. BC-3 adhesion to FN was also reduced by 67% (3.49 ± 1.04%, P=0.01) by anti-CD49d/α4, whereas BCP-1 adhesion to FN was moderately although significantly reduced by anti-CD49c/α3 (24.76 ± 15.06%, P=0.03), anti-CD49d/α4 (22.25 ± 5.80%, P=0.01) and anti-CD49e/α5 (13.76 ± 6.64%, P=0.04) mAbs, with 8%, 18% and 49% inhibition, respectively. Moreover, competing RGD peptides significantly reduced BCP-1 attachment to FN, in a dose-dependent manner, suggesting a predominant involvement of αvβ3/5. Conclusions. All PEL cell lines express the LN receptor αvβ3/5 and at least one FN receptor, mostly α4β1/β4-A4. BBG-1, BC-3 and SI-1 cell attachment to LN is predominantly αvβ3/5-VLA-6-dependent. BC-3 binding to FN is mostly α4β1/VLA-4-dependent, whereas BCP-1 binding to FN is mediated by αvβ3/5/VLA-3, α4β1/VLA-4 and αvβ3/5-VLA-5, with a predominant involvement of αvβ3/VLA-5. These integrin receptors may represent interesting targets for the development of novel therapeutic strategies for patients with PEL.

**References.**


**0357**

**MANTLE CELL LYMPHOMA CELLS EXPRESS B7 FAMILY MOLECULES AND B7-H1 EXPRESSION ARE UP-REGULATED AFTER INTERFERON-GAMMA AND LPS EXPOSURE VIA MEK-DEPENDENT PATHWAY IN MANTLE CELL LYMPHOMA CELLS**

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**Background.** Mantle cell lymphoma (MCL) is a distinct subtype of B-cell non-Hodgkin lymphomas characterized by a specific t(11;14) (q13;q21) translocation, causing over-expression of cyclin D1. Recent studies demonstrated that B7 family molecules were not only expressed on antigen presenting cells but also on various hematopoietic malignancies and solid tumors, and may play important roles in tumor immunology. Many cytokines could upregulate the expression of B7 molecules, however, the molecular mechanism of regulating expressions of B7 molecules in mantle cell lymphoma are still unknown.

**Aims.** We detect the expression of B7 family molecules in mantle cell lymphoma cells and investigate the expression of B7 family molecules in mantle cell lymphoma cells after interferon-gamma and LPS exposure and study the cell signaling pathway involved. Methods. RNA isolation, RT-PCR, quantitative real-time polymerase chain reaction, Flow cytometry, Cellular lysate preparation, Western blot analysis and Signal transduction analyses. Results. RT-PCR and flow cytometry demonstrated that MCL patients and cell lines express B7 family molecules. After interferon-gamma and LPS stimulation, B7-H1 expression were upregulated detected by flow cytometry in MCL patients and cell lines. When we knocked down TLR4, LPS stimulation did not up-regulate B7-H1 expression. Pretreatment and coinubcation of MCL cells with the MEK1/2 inhibitor U0126 reduced interferon-gamma and LPS induced B7-H1 expression, indicating that the MEK pathway was crucial for B7-H1 expression in MCL cells. To confirm that LPS and interferon-gamma induced B7-H1 expression through a MEK pathway in MCL cells, we stimulated MCL cells with LPS or interferon-gamma and analyzed the phosphorylation of ERK1/2 at different time. ERK1/2 phosphorylation were significantly up-regulated following LPS or interferon-gamma treatment. We confirmed that pretreatment of the cells with MEK inhibitor U0126 inhibited LPS or interferon-gamma induced phosphorylation of ERK1/2. Summary/Conclusions. In conclusion,

**Figure 1.**
our study demonstrated that mantle cell lymphoma cells express B7 family molecules. B7-H1 expression were up-regulated after interferon-gamma and LPS exposure via MEK-dependent pathways in MCL cells.

### Table 1

<table>
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<th>Subpopulation</th>
<th>CD45RA (%)</th>
<th>CD10 (%)</th>
<th>CCR2 (%)</th>
<th>CD19 (%)</th>
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<td>CD45RA+</td>
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### Figure 1. Functional classification (High Skp2 vs Low Skp2).

## 0358

### DECREASED NUMBER OF CIRCULATING T REGULATORY CELLS AMONG PATIENTS WITH NEWLY DIAGNOSED DIFFUSE LARGE B CELL LYMPHOMA

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**Introduction.** T regulatory cells (Tregs) are a subset of lymphocytes contributing to immune evasion by malignancies. However, in contrast to solid tumors their role in promoting diffuse large B cell lymphoma (DLBCL) has not been clearly established. So far the presence of Tregs was demonstrated in involved tissues with conflicting results with regard to prognosis. The goal of our study was to prospectively analyze the number of Tregs in peripheral blood of patients with newly diagnosed DLBCL. Results were correlated with clinical characteristics.

### Methods.** Blood samples from 19 patients with median age of 58.5 (22-79) years were collected and analyzed with the use of multiparameter flow cytometry for the presence of CD3+CD25+FoxP3+ (phenotype of “natural” Tregs) cells including subtypes characterized by expression of CD45RA (naive Tregs), HLA-DR (marker of activation), CTLA4 (marker of costimulation), CD39 (selectin P), CD62L (marker of homing to in-flamed regions). Results were compared with those achieved for 12 of costimulation), CD39 (selectin P), CD62L (marker of homing to involved areas and therefore may play a role in the pathogenesis of DLBCL.

### Results.

The absolute number of circulating Tregs, the number of Tregs in peripheral blood of patients with newly diagnosed DLBCL group. Among patients with DLBCL the number of circulating Tregs was higher for patients in good performance status (ECOG=0) compared to those with ECOG=1 or 2. 46 (23-107) x10^6/L vs. 10 (4-45) x10^6/L, p=0.004.

### Conclusions.

These results serve further investigation as candidates for new therapeutic targets.

## 0359

### DNA MICROARRAY ANALYSIS IN DLBCL DEPENDENT ON SKP2 EXPRESSION

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**Background.** The heterogeneity of diffuse large B-cell lymphoma (DLBCL) has prompted the search for new markers that can accurately separate prognostic risk groups. We previously showed in multivariate analysis that high Skp2 expression by immunohistochemical method was a strong predictor of poor outcome in DLBCL. To better characterize the molecular mechanisms, we performed the DNA microarray study on DLBCL patients. Material and Methods. To investigate which genes are aberrantly expressed in DLBCL cells, we performed cDNA microarrays and compared gene expression profiling of either group with high (n=4) or low Skp2 DLBCL cells (n=4). Results. We selected 633 genes, 311 upregulated and 322 downregulated, which showed significant differences with a P-value of <0.01 (lima). IPA (Ingenuity pathway analysis) showed clearly the network composed of cell cycle regulators, which mediate cell cycle progression during the G1/S checkpoint. A computer-assisted approach was used to procure specific molecular signalling pathways that were aberrantly expressed in DLBCL cells. Several genes related to cyclins and cell cycle regulation and to the MAPK, JAK/Stat, WNT, tumor growth factor β and Myc mediated apoptosis signalling pathways were altered in DLBCL cells when compared with Skp2 levels. In addition, MTA3 (Metastasis -associated protein 5), which was direct coexpressor WNT4 pathway, was a high average increase (1.16-lima/fold change, respectively) in high Skp2 DLBCL MTA3 are reportedly highly expressed in high-growth fraction lymphomas, such as Burkitt Lymphoma, DLBCL. Conclusion. These genes may play a significant role in the pathogenesis of DLBCL and deserve further investigation as candidates for new therapeutic targets.
instability region (MCIR). Genes within MCIR are often silenced in cancer cells by DNA rearrangements and/or by epigenetic mechanisms (methylation). This 3p21.3 region was the most frequently deleted (LOH was detected in 83% of informative cases) in human solid tumors from different tissues (576 tumors from 10 tissues were analyzed) (Petursdottir et al., 2004). EBV is associated with endemic Burkitt’s lymphoma (BL) and post-transplant lymphoproliferative disease. EBV infection leads to B-cell activation and transformation. Upon infection viral proteins induce interferon pathway, cell-surface adhesion molecules, activation antigens, chemokines and CRs (CCR6, CCR10, and CCR7). The aim of this study was to examine expression of CCR1 and CCR2 in long-time cultivated CD10+ B-cell lines both, EBV-negative (EBV-) and EBV-positive (EBV+), and also in peripheral blood (PB) circulating CD10+ B-cell subset of primary patients (prior specified diagnosis and treatment) with B-cell lymphoproliferative disorder (LPD). Twenty three B-cell lines (11 EBV+ and 7 EBV-), 3 PB B-cell lymphomas (BcL), and 2 diffuse histiocytic lymphomas (DHL) were assayed by duplex RT-PCR for CCRs (CCR1, CCR2, CCR5) from 3p21.31 region, CXCR4-CD markers (CD10, CD30, CD34, CD38, CD77), and EBV genes (EBNA1, EBNA2, LMP1) as well. Eleven cell lines (all BL) that transcribed CCR1 (among them 7 transcribed also CCR2), and PB of 8 patients were analyzed by polychromatic flow cytometry (FC), using monoclonal antibodies CD19-PerCP-Cy5.5, CD10-PE, CD191-Alexa-Fluor647 and CD192-Alexa-Fluor647. All cell lines were negative for CD34 and CCR5 transcripts, and were positive for CXCR4 transcript. In all 12 EBV- cell lines CCR2 transcript was not found, but CCR1 transcript was detected in two. On the contrary, among 11 EBV+ cell lines seven were CCR2-transcript positive and nine were CCR1-transcript positive. Notably that by polychromatic FC CCR2 was only found in about 10% of cells in 3 EBV+ cell lines, but CCR1 was present in the range of 4 - 36% in 9 out of the 11 EBV+ and in the range of 6 - 10% in two EBV-cell lines. In 8 samples of primary patients with CD10+ B-cell LPD CCR1 and CCR2 were observed on PB circulating CD10+ B-cell subset in the range of 98 - 100% and 76 - 99% respectively. Our results tentatively suggest that the lack of CCR2 in PB circulating CD10+ B-cell subset might be associated with progression of immature B-cell malignancy. Obviously, further extensive studies are necessary for the verification of our hypothesis. Petursdottir et al., Genes Chromosomes Cancer, 2004; 41:232-242.

**Background.** In patients with FL, quality of response to first-line therapy has been linked with improved survival. Additional active treatment options are required for patients with relapsed FL to improve outcomes. Rituximab is approved for relapsed/refractory FL; bortezomib has shown activity alone and in combination with rituximab in relapsed FL. The international, multicenter, phase 3 LYM3001 study compared bortezomib-rituximab with rituximab alone in patients with rituximab-naïve/-sensitive relapsed FL. Aims. This analysis was conducted to determine the impact of quality of response to treatment on outcomes. Methods. Patients with grade 1/2 measurable, relapsed FL (time to progression[TTP] ≥ 6 months for prior rituximab-containing therapy) were randomized (1:1) to five 35-day cycles of bortezomib (1.6 mg/m², days 1, 8, 15, 22, all cycles) plus rituximab (375 mg/m², days 1, 8, 15, 22, cycle 1, and day 1, cycles 2-5), or rituximab alone. All patients provided written informed consent. The primary endpoint was progression-free survival (PFS). Response/progression were assessed by an independent radiology committee using modified International Workshop Response Criteria. PFS, duration of response (DOR), TTP time to next lymphoma treatment (TTNT), and treatment-free interval (TFI) were assessed in patients achieving complete response (CR)/unconfirmed CR (CRu) (verified by bone marrow and lactate dehydrogenase), partial response (PR), or no response (NR). Results. A total of 676 patients were enrolled to receive bortezomib-rituximab (n=336) or rituximab alone (n=340). Baseline characteristics were generally well balanced between arms; median age was 57 (range 24-83)/57 (range 21-84) years, 51%/40% were male, 41%/41% had high (≥6) FLIPI score, and 43%/44% had high (≥3) FLIPI score, and 43%/44% had received prior rituximab in the bortezomib-rituximab/rituximab arms. After a median follow-up of 33.9 months, median PFS was 12.5 months with bortezomib-rituximab versus 11.0 months with rituximab (HR 0.822, p=0.039); overall response rates (CR/CRu+PR) were 63% versus 49% (p<0.001), including 25% versus 18% CR/CRu. In both arms, PFS was significantly longer in patients who achieved CR/CRu versus PR versus NR (bortezomib-rituximab: 32.6, 13.6, 4.5 months, respectively; rituximab: 33.2, 14.1, 4.7 months; p≤0.001 for all comparisons). Similarly, higher quality of response was associated with longer DOR, TTF, TTNT, and TFI in both treatment arms. Median DOR was 16.0 and 13.8 months, with 50%/32% of patients in the bortezomib-rituximab arm and 38%/23% in the rituximab arm having responses durable for 6/12 months. Median TTP was 13.5 versus 11.3 months (HR 0.808, p=0.027), median TTNT was 25.0 versus 17.7 months (HR 0.799, p=0.024), and median TFI was 17.7 versus 13.0 months, and 1-year OS rate was 90.1% versus 90.5%, with bortezomib-rituximab versus rituximab. In the bortezomib-rituximab and rituximab arms, 46%/21% of patients had grade
Background. Survival of patients with indolent lymphoma has improved in the recent decade. While it is clear that the addition of rituximab to induction chemotherapy improves survival of these patients, it is unclear which is the best chemotherapy to combine with rituximab. None of the chemotherapy regimens that had been compared in randomized controlled trials (RCTs), were superior in terms of overall survival (OS). A number of RCTs have examined the effect of bendamustine in patients with indolent lymphoma. Progression free survival (PFS) was similar or prolonged with bendamustine compared to other chemotherapy and an OS benefit has not been shown. Aims. We performed a systematic review and meta-analysis to evaluate the effect of bendamustine on the OS of patients with indolent lymphoma.

Methods. We included RCTs that compared bendamustine to other chemotherapy regimens for patients with indolent lymphoma. In December 2010 we searched The Cochrane Library, MEDLINE, LILACS, conference proceedings, and databases of ongoing trials. The primary outcome was all cause mortality. Relative risk (RR) for dichotomous data and hazard ratio (HR) for time to event data were estimated and pooled. Results. We identified 4 trials, conducted between the years 1994 and 2010 randomizing 1251 adult patients with a mean/median age of 59 - 68. The rate of patients with follicular lymphoma ranged between 40% to 52%, and mantle cell lymphoma 20% to 22% in the 3 trials that included patients with those types of lymphoma. One trial included only patients with chronic lymphocytic leukemia (CLL). The comparisons were between bendamustine, vincristine, prednisone to cyclophosphamide, vincristine, prednisone (COP); bendamustine-rituximab to cyclophosphamide, Adriamycin, vincristine, prednisone, rituximab (RCHOP); bendamustine-rituximab to fludarabine-rituximab; and bendamustine to chlorambucil. Patients treated with bendamustine had an improved OS compared to controls, RR for death 0.80; 95% CI 0.67 - 1.01. PFS was improved with bendamustine, HR 0.47; 95% CI 0.39 - 0.57. The rate of complete responses improved with bendamustine compared to controls, RR 2.31; 95% CI 1.07 - 4.60, random effects model, I2 = 88%. The rate of grade 3/4 adverse events was unaffected RR 1.21; 95% CI 0.99 - 1.48. Conclusions. This meta-analysis shows for the first time that bendamustine improves OS and PFS of patients with indolent lymphoma and CLL compared to other chemotherapy. These results should be interpreted cautiously due to the wide clinical heterogeneity of patients and treatments. Further trials of a more homogenous group should be performed to explore the role of bendamustine in various lymphoproliferative neoplasms.
was 114 days and ORR was 38% with the immediately previous line of therapy, as compared with a median PFS of 121 days and ORR of 39% for pralatrexate. Conclusions. This analysis demonstrated that patients with PTCL exhibit progressive resistance to treatment in which outcomes worsened with successive therapy. This trend was reversed with pralatrexate. Pralatrexate demonstrated higher responses and longer PFS than would be expected in a later line of therapy, thus reversing the trend of progressive resistance.

0364
DICE AS SALVAGE TREATMENT IN PATIENTS WITH RELAPSED OR REFRACTORY EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE
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Background and Aims. Recently, non-anthracycline-based chemotherapy was shown to be effective in extranodal NK/T-cell lymphoma, nasal type. Thus, a prospective phase II study was conducted in our institution to evaluate the efficacy and safety of DICE regimen in patients with untreated or relapsed disease. Here, we reported the treatment outcomes of DICE in the salvage setting. Methods. Thirty-eight patients with relapsed or refractory extranodal NK/T-cell lymphoma, nasal type were enrolled and received DICE (dexamethasone 40 mg, ifosfamide 1200 mg/m², cisplatin 20 mg/m² and etoposide 75 mg/m² on days 1 to 4) as salvage treatment. After chemotherapy, radiotherapy could be considered for patients with localized stage or residue disease if possible. Results. The median age of the patients was 38 years (range, 21 to 79). Other major patient characteristics were shown in Table 1. Of the 18 patients with stage IV disease, skin involvement was most frequent and developed in 10 patients (55.6%). In terms of prior treatment, 27 patients (71.1%) received radiotherapy and 31 patients (81.6%) had chemotherapy exposure which was mostly anthracycline-based (77.4%). The median cycles of DICE was four (range, 1 to 6). Twenty-one patients responded to DICE with an overall response rate of 55.3%. The complete and partial response rates were 23.7% and 31.6%, respectively. Radiotherapy was given to 13 patients with localized stage or residue disease after chemotherapy. Among 11 patients of them, who did not achieve complete response with chemotherapy, 6 patients (54.5%) were rendered disease-free by radiotherapy. Hematological toxicities were remarkable, but easily managed including dose-reduction in 17 patients (44.7%). All patients experienced grade 3/4 neutropenia and 10 patients (26.3%) had grade 3 neutropenic fever. The grade 3 and 4 anemia rates were 10.5% and 7.9%, respectively. Seven patients (18.4%) experienced grade 3 thrombocytopenia. Grade 3/4 non-hematological toxicities were uncommon except nausea/vomiting. There was no chemotherapy-related death. With a median follow-up of 22 months (95% CI, 8.4 to 35.2), 17 patients died and 7 of them were complicated by hemophagocytic syndrome. Two-year progression-free and overall survival (OS) rates were 42.9% and 58.7%, respectively. Sub-group analysis demonstrated that chemo-sensitivity significantly correlated with survival. Two-year OS rates for patients with complete response, partial response and no response were 77.8%, 66.7% and 35.3% (P = 0.019). Through multi-variate analysis, response status after chemotherapy was also found to be the sole independent prognostic factor (RR 1.74, 95% CI 1.15 to 2.62, P = 0.009). Conclusions. In the present study, DICE was proved to be effective with manageable toxicities in patients with relapsed or refractory extranodal NK/T-cell lymphoma, nasal type. Response after chemotherapy had a significant impact on survival outcome. Efforts need to be done in an attempt to improve chemo-sensitivity which might be achieved by integrating novel agents with DICE. e-mail address: medoncolo@gmail.com (Ye Guo, MD).

0365
LONG-TERM FOLLOW-UP OF RITUXIMAB AND INFUSIONAL CYCLOPHOSPHAMIDE, DOXORUBICIN, AND ETOPOSIDE (CDE) IN COMBINATION WITH HAART IN HIV-RELATED NON-HODGKIN’S LYMPHOMAS (NHL)
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Background. The combination of Rituximab plus chemotherapy (CT) is more effective than CT alone in the treatment of high grade NHL. Objective. To report the long-term follow-up of CDE plus Rituximab in HIV-NHL. Methods. In June 1999, we started a phase II study using infusional CDE (Cyclophosphamide 187.5 mg/m²/day, Doxorubicin 12.5 mg/m²/day and Etoposide 60 mg/m²/day) administered by continuous intravenous infusion for 4 days every 4 weeks and Rituximab 375 mg/m² i.v. on day 1. HAART was given concomitantly with CT. Results. Seventy-four patients (pts) have been enrolled. The median CD4+ cell count was 161 (range 3 to 811) and the median Performance Status was 1 (range 0-3). Diffuse large B-cell NHL was diagnosed in 72% of pts and Burkitt in 28%. Seventy per cent of pts had advanced stage (III-IV) disease and 57% of pts had an age-adjusted international prognostic index >2. Fifty-two out of 74 pts (70%) achieved a complete remission (CR), 4/7 (5%) had a partial remission and 18 pts progressed. With a median follow-up of 61 months, only 17% of CRs have relapsed and 41/74 pts are alive. The overall survival, disease free survival and time to treatment failure (TTF) at 5 years were 56%, 81% and 52%, respectively. Four cases of secondary tumours have been observed. No case of late pulmonary or cardiac toxicity has been reported. Conclusions. The combination of Rituximab and CDE in HIV-NHL treated concomitantly with HAART is very active. CR rate (70%) and TTF at 5 years (52%) are comparable to those observed in high grade NHL of the general population. Our data confirm that in HAART era a high proportion of HIV-NHL can be cured.

0366
NAVITOCXAL (ABT-263) SAFETY AND EFFICACY IN PATIENTS WITH RELAPSED OR REFRACTORY LYMPHOMA MALIGNANCIES: PRELIMINARY PHASE 2 RESULTS FROM A PHASE 1/2A STUDY
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Background. Bcl-2 family proteins are associated with tumor initiation and are frequently over-expressed in lymphoid malignancies. Navitoxal, a novel, oral, small molecule BHB mimetic with clinical activity in lymphoid malignancies, binds with high affinity (Ki ≤1 nM) and inhibits Bcl-2, Bcl-XL and Bcl-w, which regulate survival of lymphocytes, platelets and spermatocytes. Methods. This is a phase 2a safety-expansion portion of a phase 1/2a, single-agent, international study of patients with relapsed/refractory lymphoid malignancies, n1 prior chemotherapy regimen and ECOG status ≤1. The Phase 1 results have been reported previously. Following a 7 to 14-day lead-in dose-titration of 150 mg/day oral navitoxal, patients with platelet count <50,000/mm³ proceeded to
21/21-day continuous dosing at 250 mg/day on Day 1 (D1) followed by possible dose titration to 325 mg/day, based on safety data from the initial 11 patients. Safety, efficacy, and pharmacokinetics (PK) were evaluated in Arm A (relapsed/refractory follicular lymphoma [FL]) and Arm B (other indolent B-cell lymphoid malignancies). Preliminary efficacy analyses included tumor response (IWG, NCI-WG criteria) and progression-free survival (PFS). Dose-limiting toxicity (DLT) is reported for Arm A at 1/2 doses, evaluated by NCI CTCAE v3. Results. Twenty-six patients (median age 62 years, range 42-86) are enrolled in the phase 2a study. Eleven patients enrolled on Arm A (FL), and 15 on Arm B (CLL [6], SLL [2], mantle-cell lymphoma [2], lymphoplasmacytoid lymphoma [2], marginal-zone lymphoma [1], low-grade B-cell lymphoma nos [1], and transformed prolymphocytic leukemia [1]). Tumor response for patients in Arm A: 1 CRu, 4 SD, 4 PD, 2 incomplete data; and in Arm B: 6 PR, 6 SD, 1 PD and 2 incomplete data. For CLL/SLL patients in Arm B, 4 had PR (2 nodular), 2 SD, 1 PD; 1 incomplete data; and 7 had >50% reduction in absolute lymphocyte count. Overall 7 (27%) patients had objective responses. Median FFS [95% CI] for all patients was 6.4 months [8.9, not reached (NR)]; Arm A 3.0 months [1.5, 3.8]; and Arm B 6.4 months [NR, NR]. The most common navitoclax-related AEs (any grade) were diarrhea (81%), nausea (50%), and thrombocytopenia (42%); the most common Grade 3/4 AE was thrombocytopenia (51%). Two patients had severe AEs: bacterial sepsis, tumor lysis (1 each), and 7 patients had AEs (mainly thrombocytopenia) leading to dose reduction. Navitoclax exposure appeared consistent across cycles. Conclusions. Navitoclax was reasonably well tolerated with most toxicity due to on-target effects. Thrombocytopenia was predictable and manageable. Following a lead-in dose of 150 mg/day oral navitoclax, patients with platelet counts ≥ 50,000/mm3 were enrolled in cycles of 21/21-day continuous dosing at 250 mg/day/m2 D1 followed by possible dose titration to 325 mg. As observed in the phase 1 study, CLL/SLL showed the best tumor responses. In other tumor types, navitoclax should be tested in combination with other agents.

**0387 RESULTS OF A PHASE 2 STUDY OF AME-133V (LY2469298), AN FC-ENGINEERED HUMANIZED MONOCLONAL ANTIBODY, IN LOW AFFINITY FCGRIIA PATIENTS WITH PREVIOUSLY TREATED FOLLICULAR LYMPHOMA**

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**Aim**

Clinical evaluation of the safety, efficacy, and pharmacokinetics (PK) of AME-133V in patients with relapsed/refractory FL that harbors a selective reduction of B-cells during and after AME-133V treatment. Similar to rituximab, lymphocyte subset analysis showed a significant selective reduction of B-cells during and after AME-133V treatment.

**Methods**

A Phase 2 study was conducted to assess the safety and efficacy of AME-133V in patients with relapsed/refractory follicular lymphoma (FL) who expressed a low affinity variant of FcγRIIa. The Phase 1 dose-escalation component of a Phase 1/2 clinical trial of AME-133V at doses ranging from 2 - 375 mg/m2 demonstrated that AME-133V was safe and tolerable at all dose levels tested, and 375 mg/m2 was chosen for further assessment. Aims. The aim of the Phase 2 component of this study was to determine the safety and tolerability of four, weekly infusions of AME-133V at 375 mg/m2, and to determine the pharmacokinetic (PK) profile, objective response rate, and duration of response in patients who are F-carriers in one or both alleles that encode amino acid position 158 in the FCγRIIa gene. Methods. After obtaining informed consent, 50 patients were enrolled. Based on the Phase 1 dose escalation results, AME-133V was administered at the highest previously tested dose of 375 mg/m2 intravenously every week for 4 weeks. Six patients were treated at 375 mg/m2 during dose-escalation and are included in this analysis as pre-specified in the protocol. Safety, PK, response, and progression free survival were assessed. Response was also assessed by an independent central reviewer. Results. The median age was 61 (39-83) years and the median number of prior therapies was 2 (1 - 9). The majority of patients (54%) received prior rituximab (median number of doses was 8 (0-24)). There were 22 patients with Va/F and 28 with Va/F/FeRκIIa allergies. Forty-five patients had ≥1 prior lymphoma treatment; 14/45 (31%) were reported to have had a dose-limiting toxicity (DLT). Two patients died during the study; one due to life-threatening oesophageal achalasia and one due to aspiration pneumonia. Investigator-assessed responses were observed in 15 (30%) patients, including 4CRs and 3 CRs. The centrally assessed response rate was 32%. The median progression free survival (PFS) was 8.3 months as assessed by the investigators. The pharmacokinetic profile of AME-133V was similar to rituximab. Lymphocyte subset analysis showed a significant and selective reduction of B-cells during and after AME-133V treatment. Summary/Conclusions. AME-133v was safe and well tolerated at the recommended Phase 2 dose of 375 mg/m2. Clinical responses, including 14% with CR/CRu, were observed. These data demonstrate that AME-133V is active in previously-treated FL patients who are F-carriers at amino acid position 158 in the FCγRIIa gene.
reduction, and an additional 3 patients had a dose reduction only. At the time of interim analysis, end of treatment response were 94% (8 CR, 8 PR) in G-CHOP arm and 93% (14 CR and 12 PR) in G-FC arm. Conclusion. These preliminary results indicate that G-CHOP and G-FC combinations may be delivered safely and are highly effective treatments in patients with relapsed FL. Importantly, G-CHOP can be delivered at the pre-specified three-weekly intervals without need for dose reductions or delays. GA101 is now being investigated in first-line patients in combination with CHOP and bendamustine.

Table 1.

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0369 RESOLUTION OF MALIGNANT CUTANEOUS LESIONS WITH BRENTUXIMAB VEDOTIN (SGN-35) IN PATIENTS WITH RELAPSED OR REFRACTORY SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMA

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Background. Brentuximab vedotin (SGN-35) is an anti-CD30 antibody conjugated to the highly potent antimicrotubule agent, monomethyl auristatin E (MMAE), by a plasma-stable linker. Brentuximab vedotin binds to CD30 on the cell surface, internalizes, and releases MMAE in the cytoplasm, disrupts the microtubule network within the cell, induces cell cycle arrest, and results in apoptotic death of the CD30-expressing tumor cell. In a phase 2 study of brentuximab vedotin in 58 patients with relapsed or refractory systemic anaplastic large cell lymphoma (sALCL), an objective response rate of 88% was observed by independent review. Aims. To describe the experience with brentuximab vedotin in relapsed or refractory sALCL patients with malignant cutaneous lesions who participated in a phase 2, single-arm, multicenter study. Methods. Brentuximab vedotin 1.8 mg/kg was administered every 3 weeks as a 30 minute outpatient IV infusion for up to 16 cycles of treatment. Informed consent was obtained for all patients. Determination of antitumor efficacy was based on objective response assessments by an independent review facility according to the Revised Response Criteria for Malignant Lymphoma (Cheson 2007). Resolution of cutaneous lesions was assessed by the investigator. Results. 15 patients with relapsed or refractory sALCL who participated in the phase 2 study had malignant cutaneous lesions at baseline. Among these patients, the median age was 57 years (range 33-70), and ECOG performance status was 0 or 1. Median number of prior therapies was 2 (range 1-5) and 4 patients (27%) had an autologous stem cell transplant prior to the study. The majority of patients (80%) had ALK-negative disease. Complete resolution of malignant cutaneous lesions was achieved in 93% of patients (14 of 15) with a median time to resolution of all lesions of 4.9 weeks (range 2.6-36). Objective responses were achieved by all patients (12 CR, 3 PR). Median duration of objective response was not reached (range 0.3+ to 45.3+ weeks). At the time of the analysis, the median follow up in the study was approximately 6 months. Patients received a median 7 cycles of treatment (range 1-16), with 4 remaining on treatment at the time of the analysis. The most common adverse events (≥30%) of any grade among the 15 patients were diarrhea, pyrexia, constipation, nausea, peripheral sensory neuropathy, and decreased appetite. Adverse events ≥ Grade 3 that occurred in more than 1 patient were neutropenia (4 patients), constipation, pyrexia, and asthenia (2 patients each). Summary/Conclusions. In a phase 2 trial of brentuximab vedotin in relapsed or refractory sALCL, local and systemic responses were observed among 15 patients who had malignant cutaneous lesions at baseline: 93% of patients had complete resolution of cutaneous lesions and 100% had objective responses. Adverse events were manageable, and the safety profile was comparable to that observed among patients without cutaneous involvement. These results warrant further study of brentuximab vedotin in patients with CD30-positive cutaneous and systemic lymphomas. A trial of brentuximab vedotin in patients with CD30-positive cutaneous T-cell lymphomas (CTCL) is planned.

0370 PHASE II STUDY OF INTRATEHICAL LONG ACTING LIPOSOMAL CYTARABINE IN THE PROPHYLAXIS OF LYMPHOMATOUS MENINGITIS IN HIV-RELATED NON-HODGKIN’S LYMPHOMA

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Background. Around 5% of patients (pts) with aggressive non-Hodgkin’s lymphoma (NHL) develop central nervous system (CNS) progression or relapse during the course of their disease. Pts with HIV-related NHL often develop CNS progression despite the use of adequate prophylaxis. Liposomal cytarabine has shown a significant activity in lymphomatous meningitis but there are limited data in the prophylactic setting. Methods. Since May 2006, we are running a prospective phase II study of intrathecal liposomal cytarabine at the dose of 50 mg in 48 pts with HIV-NHL with the aim to evaluate the feasibility and activity of this drug in the prevention of lymphomatous meningitis. Results. Forty-two pts were males and the median age was 44 years (range 18-69). As far as the histological subtype of NHL, 47% of pts had a diffuse large B-cell (DLBCL) NHL and 40% Burkitt NHL. Stage III-IV was diagnosed in 80% of pts and 68% of DLBCL were age-adjusted IPI 2 or more. An extramedullary involvement was diagnosed in 70% of pts (gastrointestinal 30%, bone 27%, spleen 10%, liver 22%, bone marrow 17%). Liposomal cytarabine was well tolerated with headache grade 1 to III being the most frequent side effect in only 32% of pts. Less common toxicity (all grade 1) included cortical changes (4%), fever (2%), vomiting (2%), hypertension (2%), chills (2%). With a median follow up of 15 months, only one pt (3%) with Burkitt lymphoma developed a combined systemic and meningeal relapse. Moreover, in our experience compared to the previous study, we used methotrexate as practical use in 426 HIV-NHL with a meningeal progression or relapse of 14% (p=0.09). The use of a liposomal formulation allowed to significantly reducing the number of lumbar injections in comparison to the standard schedules (approximately of 50%) with an improvement of quality of life of pts and with a reduction of professional exposure risk for health care staff. Conclusions. In this first prospective study on prophylaxis of lymphomatous meningitis in HIV-NHL reported in the literature, liposomal cytarabine seems safe and active and it reduces of approximately 50% the number of lumbar punctures and exposure risk for health staff as well.

0371 LENALDIDIOMIDE PLUS RITUXIMAB-CHOP21 IS SAFE AND EFFECTIVE IN ELDERLY UNTREATED DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): RESULTS OF PHASE I PART OF REAL07 TRIAL OF ITALIAN LYMPHOMA FOUNDATION (FIL)

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Background. R-CHOP21 is the standard treatment in newly diagnosed DLBCL of the elderly, however 30-40% of the patients failed.
Lenalidomide monotherapy in relapsed/refractory DLBCL was tested, with promising results; preclinical studies demonstrated a synergism with Rituximab. On these basis, FIL is running a prospective multicenter dose finding phase I-II trial with the aim to evaluate toxicity and efficacy of Lenalidomide plus R-CHOP21 (LR-CHOP21) for elderly untreated DLBCL patients (clinicaltrials.gov, NCT00907348). The primary endpoint for the phase I part of the study is the maximum tolerated dose of Lenalidomide for days 1-14 at the established dose level. Phase I was planned to define the Maximum Tolerated Dose (MTD), that is the dose that achieves a DLT in 33% of patients. Evaluation was planned after three LR-CHOP21 courses. The study was designed with the Continual Reassessment Method (CRM), a Bayesian model that uses, as dose allocation rule of the sequentially incoming patients, the re-estimated probability of toxicity based on the results obtained for the patients already observed. Four doses of Lenalidomide were tested: 5, 10, 15 and 20 mg. At the end of each cohort, the dose level associated with an updated DLT probability closest to 33% was recommended to be administered to the next cohort. Results. From May 2008 to February 2010, 21 patients were enrolled in the phase I part of the study. Clinical characteristics were: median age 68 (61-77); stage III/IV 81%; PS 0 > 1 81%; IIH/H IPI risk 52/24%. Patient allocation to Lenalidomide dose (on days 1-14 of each LR-CHOP21 course) was: 5 mg/day in nine patients, 10 mg/day in nine patients, 15 mg/day in nine and 20 mg/day in three. DLTs in the first three courses of LR-CHOP21 were recorded in seven patients; according to CRM, these events determined Lenalidomide 15 mg/die as the MTD in association to R-CHOP21. Of 115 LR-CHOP21 courses performed in the series of 21 patients, hematological toxicity was mild: grade III/IV neutropenia occurred in 10% of courses, anemia in 4% and thrombocytopenia in 28%. Extra-hematological toxicities were moderate: grade IV increase of CPK in one patient, grade III cardiac in one, grade III neurological in three and grade III infections in four (two pneumonias, one febrile neutropenia with diarrhea and one diarrhea). At the end of the phase II part of the trial I patients were defined to test the efficacy of 15 mg of Lenalidomide in association with R-CHOP21.

0372 PRIMARY MEDIASTINAL LARGE B CELL LYMPHOMA (PMBL). LONG TERM RESULTS AND LATE TOXICITY IN PATIENTS TREATED WITH MACOP-B WITH OR WITHOUT RITUXIMAB PLUS INVOLVED MEDIASTINAL RADIATION THERAPY

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Background. Primary mediastinal large B cell lymphoma is a distinct subtype of diffuse large B cell lymphomas that is more common in younger females. The combination of chemotherapy (CHT) together with involved field radiation therapy (IFRT) is considered the standard treatment. In the pre-Rituximab era third generation regimens such as MACOP-B have improved survival in PMBLS patients (pts) over CHOP, but the introduction of Rituximab has abrogated this difference. The real need of consolidation mediastinal IFRT is still debated in view of the risk of secondary cancers and cardiac complications. We report the long-term results on a large series of PMBL pts treated at a single center. Method. 107 patients (pts) with PMBL were treated between June 1991 and September 2006 at our institute; 80 pts had stage II and 27 stage IIE-IV; 75% had elevated LDH; bulky disease was present in 95 pts including 58 (55%) with clinical evidence of superior vena cava obstruction. Median age was 34 yrs (15-61) and 71% were females. The ally/day in nodal; 10 mg/pt in 47. Ninety-five pts were treated with the standard MACOP-B regimen and 15 pts with a R-CHOP21/MACOP-B regimen since March 2004. Overall, 101/107 pts (94%) received IFRT at a dose of 30-36 Gy. The response was evaluated in all pts at the end of CHT and of IFRT. Results. At the end of the program, a CR/CRu was obtained in 76 pts (71%), a PR in 23 (21%), NR 1(1%), while 7 pts were not evaluable (6 pts received an early intensification for progressive disease and 1 died for CHT related toxicity). At the end of the program: 14 PR pts obtained a CR/CRu after IRFT with an overall CR/CRu rate of 89%; 9 pts relapsed within 10 months and 4 of them died of progressive disease. After a median follow-up of 111 months (1-238) the 10-yrs OS, PFS and EFS were 88%, 85% and 83% respectively. No statistically significant difference in terms of PFS and OS and toxicity was recorded for pts treated with or without Rituximab. Patients with an IPI 0-1 had a significantly better PFS (p=0.020) and OS (p=0.015). In our cohort of pts, 1/107 developed a secondary cancer (acute myeloid leukemia) after 164 months from the end of therapy and no breast cancer occurred. Four of 107 pts presented late severe cardiotoxicity (3 congestive heart failures and 1 arhythmic sudden death). Conclusions. This is the largest reported series of pts with PMBL treated with a uniform strategy at a single center. MACOP-B +/- Rituximab plus IFRT is highly effective and devoid of severe long-term toxicities. Future randomized trials should evaluate the real need of a mediastinal IFRT in pts who obtain a PET-negative CR after a R-chemotherapy regimen to reduce unexpected late toxicities.
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RESULTS FROM A PHASE 3 STUDY
TREATMENT WITH BORTEZOMIB PLUS RITUXIMAB OR RITUXIMAB ALONE IN PATIENTS WITH RELAPSED FOLLICULAR LYMPHOMA (FL): PATIENT-REPORTED OUTCOMES DURING AND FOLLOWING TREATMENT WITH BORTEZOMIB-rituximab resulted in improved progression-free survival, response rates, and other long-term outcomes versus rituximab alone in patients with relapsed FL in the international, multicenter, phase 3 LYM3001 study. Assessment of PROs was an exploratory endpoint. Aims. To evaluate changes from baseline in PRO scores during/following treatment with bortezomib-rituximab or rituximab using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30). Methods. Patients were randomized to receive five 5-week cycles of bortezomib-rituximab (N=336) or rituximab (N=340), as previously described (Coiffier et al, ASH 2010). All patients provided written informed consent. The primary endpoint for the PRO analyses was the Global Health Status (GHS) subscale of EORTC QLQ-C30; other endpoints included the other subscales, comprising five functional scales, three symptom scales, and six single items. Patients completed EORTC QLQ-C30 at baseline, on day 1 of each cycle, at the end-of-treatment visit, and every 10 weeks post-treatment/pre-progressive disease (PD). Results. 335 and 333 patients in the bortezomib-rituximab and rituximab arms, respectively, provided evaluable EORTC QLQ-C30 data at baseline; as expected, these numbers decreased over time, to 293/289 at the end of treatment, and 23/21 at end of data collection. At baseline, observed mean GHS scores were 62.94 and 63.11 in the bortezomib-rituximab and rituximab arms, respectively, provided evaluable EORTC QLQ-C30 data at baseline; as expected, these numbers decreased over time, to 293/289 at the end of treatment, and 23/21 at end of data collection. At baseline, observed mean GHS scores were 62.94 and 63.11 in the bortezomib-rituximab and rituximab arms, respectively, changes are shown in the figure. For analyses of changes from baseline and comparisons between arms, a joint modeling approach was used to address the potential issue of non-random missing values; values deviate from observed means due to incorporation of informative dropout and repeated measures. For bortezomib-rituximab, mean GHS was not statistically different from baseline through week 25 (end of treatment) and statistically improved at weeks 30-120; mean changes ranged from 3.57 at week 30 (mean 66.02) to 6.55 at week 80 (mean 69.00). With rituximab, mean GHS was statistically improved from baseline at weeks 5-120; mean changes ranged from 1.95 at week 5 (mean 64.92) to 7.91 at week 70 (mean 70.89). Between-group comparisons showed significantly lower mean scores with bortezomib-rituximab versus rituximab at weeks 10 (mean change -0.69 vs 2.78), 15 (mean change -1.44 vs 2.75) and 20 (mean change -1.98 vs 1.95); from week 25 there were no significant differences between arms. Changes from baseline and differences between arms were generally not sufficiently large to be considered clinically meaningful (≥5 points). Findings for changes in functional scores were similar to those for GHS. Minimal differences between arms in symptoms and side-effect scales were consistent with the adverse event profile of bortezomib. Conclusions. Rituximab resulted in slightly better PRO scores compared with bortezomib-rituximab during treatment, reflecting additional adverse events associated with bortezomib. However, post-treatment PRO scores were similar between arms and slightly higher compared to baseline. Differences/changes from baseline were not clinically significant.

0376

A NEW PROGNOSTIC SCORING MODEL FOR PERIPHERAL T-CELL LYMPHOMA, NOT OTHERWISE SPECIFIED
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Background. Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) comprises a clinically and histopathologically heterogeneous group of lymphomas which do not fit into the definition of any other identified subtype of PTCLs. Most of the cases are characterized by aggressive behavior and a dismal prognosis. Aim. To assess the clinical backgrounds and the prognosis of PTCL-NOS patients, the Hokkaido Hematology Study Group (HHSG) conducted a multicenter retrospective survey in Hokkaido, Japan. Methods. We reviewed 508 mature T-cell and NK-cell neoplasms diagnosed according to the 4th edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues at the HHSG, which includes 30 hematology/oncology or pediatrics departments of 23 institutes, from...
January 2002 to December 2009. One hundred seven (21%) PTCL-NOS patients were included and further analyzed with regard to their clinical backgrounds, treatments, remission or relapse rates, survival, and prognostic factors. The overall survival (OS) and progression free survival (PFS) were estimated using the Kaplan-Meier method and compared by a log-rank test. The risk factors at diagnosis for OS were evaluated by a univariate analysis and in a multivariate analysis by the Cox proportional hazards model. Results. The median follow-up of the patients was 24 months (range 1-95). The patients included 70 males and 37 females with a median age of 67 years (range 9-94). Chemotherapy (ChT) was selected in 90% (96/107) patients as the primary treatment. CHOP-like regimens were chosen for 91% (96/96) of patients as the primary ChT. A total of 48 (52%) of the 92 evaluable patients achieved a CR after the primary treatment, in which 46% (22/48) relapsed. The estimated 5-year OS and -PFS of all patients was 85%, and 28%, respectively. The risk factors at diagnosis associated with OS by the univariate analysis were age>60 (p=0.027), presence of B-symptom (p=0.006), an advanced clinical stage (p=0.002), a high LDH level (p=0.007), dL2<2000μ/ml (p=0.001), platelets<10x104/μl (p=0.002), lymphopenia (p=0.040), bulky disease (p=0.042), and bone marrow involvement (p=0.013). Extraneous involvement sites>1 (p=0.116) and a poor performance status (p=0.119) were not significant risk factors for a decreased OS. In a multivariate analysis, three independent risk factors for OS bulky disease (hazard ratio; HR=5.324, p=0.019), age>60 (HR=3.015, p=0.025), and platelets<10x104/μl (HR=3.999, p=0.036), were identified. Three risk groups for OS were defined by the numbers of these 3 risk factors: score 0, low risk; score 1, intermediate risk; score 2-3, high risk. The OS curves of the PTCL-NOS patients were more significantly stratified into three risk groups by using our scoring model (p=0.0005, Figure 1), compared to the results obtained by using the prognostic index for the PTCL-unspecified (PIT) scoring model (p=0.019). Conclusions. We demonstrated that the OS for PTCL-NOS patients was clearly stratified into 3 risk groups according to new prognostic scoring model by using three risk factors: bulky disease, age>60, and thrombocytopenia. Although further verification by a prospective analysis is needed, these findings may provide valuable information to help predict the prognosis or to select effective therapeutic strategies for PTCL-NOS patients.

0377 COMPARISON OF PROGNOSTIC MODELS FOR PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA: THE NEED FOR A REVISION IN THE RITUXIMAB ERA

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Background. Several revisions of International prognostic index (IPI) have been proposed for patients with diffuse large B cell lymphoma (DLBCL) after the introduction of rituximab. Expanding evidence suggests that baseline absolute lymphocyte count (ALC) is also an independent factor for outcome prediction. Aims. We investigated the optimal prognostic model for these patients in the rituximab era. Methods. The study enrolled 274 consecutive patients with DLBCL receiving first-line CHOP-based chemotherapy with rituximab between 2003 and 2009. Five factors within IPI and ALC were entered for Cox regression analysis. Overall survival (OS) was calculated for different risk groups of models. Efficacy of models was compared by the value of Akaike information criterion (AIC). Results. Revised IPI (R-IPI) and ALC/R-IPI, but not IPI, were informative to discriminate between different risk groups of patients with DLBCL. In multivariate analysis for individual factors of the prognostic models, however, only performance status > 1 (odds ratio (OR) 3.59), Ann Arbor stage III or IV (OR 2.24), and ALC less than 1x109/L (OR 2.75) remained their significance. A modified score based on the three factors divided patients into four risk groups and the 5-year OS rate was 81%, 74%, 56%, and 9% respectively. By comparing AIC values in the Cox proportional hazards analysis, the modified 3-factor model had superior prognostic value followed by ALC/R-IPI, R-IPI and IPI scores. Conclusions. Addition of the novel factor, ALC, interacts with other established factors in outcome prediction for DLBCL. Development of a new score is needed for a better risk stratification in the rituximab era and would be helpful in the design of future clinical trials. The proposed 3-factor model should be validated in large scale studies.
0379 HIGH RESPONSE RATE IN SPLENIC MARGINAL ZONE LYMPHOMA IN PATIENTS TREATED WITH RITUXIMAB, EITHER AS MONOTHERAPY OR IN COMBINATION WITH CHEMOTHERAPY

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Background. Splenic marginal zone lymphoma (SMZL) is an uncommon indolent B-cell non-Hodgkin’s lymphoma usually presenting with marked splenomegaly and bone marrow (BM) and/or peripheral blood (PB) involvement. Splenectomy has been the treatment of choice in symptomatic patients. Systemic treatment is required in patients with widespread disease, who are at high risk from surgery, or who have relapsed after splenectomy. Rituximab has shown encouraging results in SMZL, with sustained responses. Aims. To assess, retrospectively, response to treatment, toxicity and survival after rituximab in SMZL. Methods. Twenty-nine patients from two different centers, diagnosed with SMZL between 1982 and 2011, received one or more treatments with rituximab. Eighteen patients received rituximab alone and 20 combined with chemotherapy. Thus 9 patients received each of these sequentially, to improve response, with or without interim relapse. The median age at diagnosis was 62 years (range 37-89 years); the male:female ratio was 2:3; B symptoms were present in 12 patients; ECOG performance status was 0-2 in 28/29 patients. All presented with splenomegaly, with involvement of the BM in 27 patients, PB in 24, lymph nodes in 11 and extranodal involvement in 7 patients. Diagnosis was made according to the WHO 2008 classification by spleen histology (n=12), BM histology (n=19), PB morphology (n=12) and immunophenotype (n=13). Rituximab monotherapy was administered at 375 mg/m2/weekly x4 weeks. In combination with fludarabine-based regimens (n=14), or other regimens including CHOP (n= 6), rituximab was administered on day 1 of each cycle. Responses were assessed according to the Response Criteria Guidelines for SMZL (Matutes, Leukemia 2008). Toxicity was graded according to the CTCAE v3.0. The Fisher exact test was used to compare best responses between groups. Survival was estimated using the Kaplan-Meier method. Results. All patients responded to rituximab therapy; however, only a few active drugs suitable for salvage therapy exist and studies addressing new drugs and combinations in relapsing patients should be encouraged. This, patients with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy were treated with a combination of rituximab, ifosfamide and etoposide (R-IE regimen) at nine Italian centres. The choice of the drugs was based on their capability to cross the blood-brain barrier and their efficacy to treat extra-CNS aggressive lymphomas. Aims. To evaluate feasibility and activity of R-IE chemoimmunotherapy regimen in patients with relapsed or refractory PCNSL. Methods. HIV-negative patients with ≤75 years old, ECOG PS ≤3 with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy were considered. R-IE regimen consisted of four courses of rituximab 375 mg/m2 day 0; ifosfamide 2 g/m2/d days 1-3; etoposide 250 mg/m2/day 1. Results. Twenty patients (median age 60 yrs, range 39-71; M/F ratio: 1.2) were treated with R-IE, as second-line treatment in 15 patients, as third line in three and as fourth line in two patients. Thirteen patients had refractory PCNSL (progressed during previous treatment) and seven had relapsing disease. Ten patients had received whole-brain radiotherapy as part of previous treatments. Fifty-one (64%) of the 80 planned courses were actually delivered. R-IE was interrupted in 14 patients due to lymphoma progression (n=12), toxicity (n=1) and patient’s refusal (n=1); treatment is ongoing in one patient. G4 hematologic toxicity (neutropenia 50%; thrombocytopenia 25%; anemia 15%) was common but manageable; no G4-nonhematologic toxicity was observed; one patient died of pulmonary aspergillosis. Response after R-IE was complete in six patients and partial in one (ORR= 35%); 95% CI: 14%-56%), with a median response duration of 11+ months (4+ - 19+). Five responsive patients successfully collected autologous stem cells after the 2nd course of R-IE, and three of them received consolidation with BCNU 400 mg/m2 day 6; thiopeta 5 mg/kg days 5 - 6 and ASCT day 0. At a median follow-up of 11 months, no responder experienced relapse, while 12 patients experienced lymphoma progression, with a 2-yr PFS of 23% ± 11%. Nine patients are alive, nine died of lymphoma, one of pulmonary aspergillosis and one patient died of neurological impairment while in remission, with a 2-yr OS of 50% ± 12%. The number of previous lines of treatment, prior irradiation and relapsed or refractory disease did not influence response nor survival rates. Conclusions. R-IE is a feasible and active combination for patients with relapsed or refractory PCNSL. This regimen allowed autologous stem cell collection, and consolidation with high-dose chemotherapy supported by ASCT resulted in long-term remission.

0380 SALVAGE CHEMOTHERAPY WITH RITUXIMAB, IFOSFAMIDE AND ETOPOSIDE (R-IE) IN PATIENTS WITH PRIMARY CNS LYMPHOMA RELAPSED OR REFRACTORY TO HIGH-DOSE METHOTREXATE-BASED CHEMOTHERAPY

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Background. Conventional upfront high-dose methotrexate (HD-MTX)-based chemotherapy ± radiotherapy is associated with a high complete remission rate in immunocompetent patients with primary CNS lymphoma (PCNSL), however, 35-60% of responsive patients experience relapse within a few months and an additional 10-15% is refractory to primary chemotherapy. Often, salvage therapy results in a second remission with consequent symptomatic and survival improvement; however, only a few active drugs suitable for salvage therapy exist and studies addressing new drugs and combinations in relapsing patients should be encouraged. This, patients with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy were treated with a combination of rituximab, ifosfamide and etoposide (R-IE regimen) at nine Italian centres. The choice of the drugs was based on their capability to cross the blood-brain barrier and their efficacy to treat extra-CNS aggressive lymphomas. Aims. To evaluate feasibility and activity of R-IE chemoimmunotherapy regimen in patients with relapsed or refractory PCNSL. Methods. HIV-negative patients with ≤75 years old, ECOG PS ≤3 with PCNSL relapsed or refractory to HD-MTX/HD-cytarabine-based chemotherapy ± radiotherapy were considered. R-IE regimen consisted of four courses of rituximab 375 mg/m2 day 0; ifosfamide 2 g/m2/d days 1-3; etoposide 250 mg/m2/day 1. Results. Twenty patients (median age 60 yrs, range 39-71; M/F ratio: 1.2) were treated with R-IE, as second-line treatment in 15 patients, as third line in three and as fourth line in two patients. Thirteen patients had refractory PCNSL (progressed during previous treatment) and seven had relapsing disease. Ten patients had received whole-brain radiotherapy as part of previous treatments. Fifty-one (64%) of the 80 planned courses were actually delivered. R-IE was interrupted in 14 patients due to lymphoma progression (n=12), toxicity (n=1) and patient’s refusal (n=1); treatment is ongoing in one patient. G4 hematologic toxicity (neutropenia 50%; thrombocytopenia 25%; anemia 15%) was common but manageable; no G4-nonhematologic toxicity was observed; one patient died of pulmonary aspergillosis. Response after R-IE was complete in six patients and partial in one (ORR= 35%); 95% CI: 14%-56%), with a median response duration of 11+ months (4+ - 19+). Five responsive patients successfully collected autologous stem cells after the 2nd course of R-IE, and three of them received consolidation with BCNU 400 mg/m2 day 6; thiopeta 5 mg/kg days 5 - 6 and ASCT day 0. At a median follow-up of 11 months, no responder experienced relapse, while 12 patients experienced lymphoma progression, with a 2-yr PFS of 23% ± 11%. Nine patients are alive, nine died of lymphoma, one of pulmonary aspergillosis and one patient died of neurological impairment while in remission, with a 2-yr OS of 50% ± 12%. The number of previous lines of treatment, prior irradiation and relapsed or refractory disease did not influence response nor survival rates. Conclusions. R-IE is a feasible and active combination for patients with relapsed or refractory PCNSL. This regimen allowed autologous stem cell collection, and consolidation with high-dose chemotherapy supported by ASCT resulted in long-term remission.
0381

WEAKL INFUSION OF RITUXIMAB AND BORTEZOMIB IS EFFECTIVE AND SAFE IN RELAPSED/REFRACTORY INDOLENT AND MANTLE CELL LYMPHOMA: LONG TERM ANALYSIS OF A PHASE II TRIAL OF ITALIAN LYMPHOMA FOUNDATION (FIL)


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Background. Gene-profiling studies demonstrated a constitutive activation of the NFκB signalling pathway in Mantle Cell Lymphoma (MCL) and Marginal Zone Lymphoma (MZL). Bortezomib, an inhibitor of the proteasome, is effective in relapsed MCL and it is synergistic with Rituximab to enhance apoptosis and NFκB depletion. On these basis, the FIL conducted a phase II multicenter study to evaluate safety and efficacy of Bortezomib in relapsed/refractory non-follicular Lymphoma (Linfocytic, LL and MZL) and MCL, not eligible to high dose chemotherapy with stem cell transplantation. Patients and Methods. The study was a prospective phase II non randomized trial, designed on Simon two-stage Optimal Design. Primary end-point was to obtain an Overall Response Rate (ORR) > 40%. A central histological revision was planned in all the patients at the enrollment. Inclusion criteria were: 18-75 years, relapsed/refractory LL, MZL, MCL after 1-4 lines. Treatment schedule was: one course of 1.6 mg/kg Bolus Bortezomib in combination with standard 375 mg/m2 Rituximab on days 1, 8, 15, 22 followed by two courses of four weekly intravenous bolus of Bortezomib alone, patients with complete (CR), partial remission and stable disease at the intermediate evaluation were planned to be given three further courses with the same schedule. Results. From September 2006 to March 2008, 55 patients were enrolled and six were excluded at central histological revision. Clinical characteristics were: median age 68 (50-74); 16 LL, 8 MZL, 25 MCL; 42 stage III/IV, 33 bone marrow involvement. Thirty-eight patients were at third or fourth relapses, 34 Rituximab pretreated; 21 had refractory disease. ORR was 53% (CR 26.5%); no response was 43% and 4% off therapy. ORRs by clinical subgroup were: LL 37%, MZL 50%, MCL 64%; Rituximab pretreated 62%, Rituximab naïve 33%; relapsed 64% and refractory 38%. With a median follow-up of 26 months, median Overall Survival was not reached and median Progression Free Survival (PFS) was 9.9 months (95% CI: 4.8 - 18.3). Median PFS by histology was: 4.8 (95% CI: 4.1 - 8.9) for LL, 18.3 (95% CI: 5.3 - 29.9) for MCL and 9.9 months (95% CI: 2.4 - not reached) for MZL. Thirty patients completed the treatment and 233 courses were delivered (median: 4.7 courses/patient); 19 patients did not because of no response in 13, adverse events in five, with only one toxic death due to interstitial pneumonia. Of 233 courses performed, hematological toxicity was rare: grade III/IV neutropenia in 5% and thrombocytopenia in < 2% of all courses. Grade III/IV CTC non-hematological toxicities were: neurotoxicity grade III in four patients (all completely recovered) and infections in eight patients (viral reactivation, bacterial pneumonia and meningitis). Conclusions. Weekly infusion of Bortezomib in combination with Rituximab is effective and safe in relapsed/refractory indolent and MCL, also in Rituximab pretreated patients. Data demonstrated that this schedule is effective mainly in MZL and MCL.

0382

PGP IS EXPRESSED IN MESENCHYMAL STEM CELLS AND CAN BE IMPLEMENTED FOR THEIR ISOLATION

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Background. Human mesenchymal stem cells (MSCs) are multipotent stem cells that can differentiate in vitro and in vivo into various cell types. Therefore, MSCs exhibit high potential for therapeutic applications. MSCs are found in various tissues, including bone marrow (BM) and umbilical cord blood (UCB). AIMS. It is commonly accepted that BM- and UCB-derived plastic- adherent cells (mesenchymal-like stem cells) are very heterogeneous, comprising only a small subset of authentic stem cells. These cells are currently identified through a combination of poorly defined physical, phenotypic and functional properties. Thus a more comprehensive view of the MSCs identity and characteristics is urgently needed. METHODS. UCB and BM-derived mononuclear cell populations were grown in culture for 1 week. The plastic adherent cells were detached by EDTA. Cells were labeled with anti-CD105-APC and with MRK16 antibody against Pgp and secondary rabbit anti-mouse antibody PE. The CD105/Pgp subsets were sorted by Fluorescence-activated cell sorter. These subsets were analyzed for: (1) formation of Colony Forming Unit-Fibroblasts (CFU-Fs); (2) expression of a panel of surface markers of MSCs and lack of expression of hematopoietic markers; and (3) differentiation potential toward osteocytes and adipocytes under specific in vitro differentiating conditions. RESULTS. In this study, we demonstrate that a MSCs from human UCB and BM can be identified and isolated based on a single known surface antigen (e.g., CD105) and coexpression of the ABC transporter P-glycoprotein (Pgp). Among the plastic adherent MSCs-like cells, only the CD105+/Pgp+ subset demonstrates all the criteria for MSCs. These include long survival; fibroblast-like (CFU-F) morphology; expression of a panel of MSCs positive markers (CD105, CD44, CD73, CD90, CD271) but not expression of the hematopoietic stem cells’ markers (CD34, CD45). Moreover, only the CD105+/Pgp+ cells can differentiate into osteogenic and adipogenic in the presence of specific supplements. Upon these differentiation pathways, the Pgp is down regulated, suggesting that Pgp is a novel marker for identifying MSCs. Functional blocking of Pgp activity by Pgp-specific inhibitor AM151, significantly augments the adipogenic differentiation pathway of the MSCs but has apparently no effect on the osteogenic differentiation pathway. CONCLUSIONS. This study indicates that overexpression of Pgp is characterized for genuine MSCs and can be utilized to isolate the relatively small subset of multipotent MSCs from the heterogeneous adherent cell populations of BM and UCB.

0383

ENHANCED ADHESION & MIGRATION AND INDUCTION OF PYK2 COMPLEX FORMATION IN NB4 AND K562 CELLS FOLLOWING ATRA AND IMATINIB TREATMENT

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Background/Aims. All-trans-retinoic-acid (ATRA) and imatinib (IM) are targeted therapies used for acute promyelocytic leukemia (APL) and chronic myeloid leukemia (CML), respectively. Despite improved prognosis, ATRA and IM administration has been associated with extramedullary disease (EMD) occurrence. We postulate that, like in the myeloid malignancies, changes in Traction and adhesion potential may enable leukemic cells to inhabit extramedullary sites. Focal adhesion complexes linking between extracellular matrix and the cell cytoskeleton are likely to play an important role in these processes. Pyk2 is a tyrosine kinase highly expressed in hematopoietic cells. Proteins such as paxillin, talin, vinculin, etc, interact with Pyk2 creating the Pyk2 associated signaling complex at focal adhesion sites. These complexes participate in adhesion and migration processes and may be involved in EMD development. Our objectives are to identify the molecular changes associated with ATRA and IM administration, define their...
effect on adhesion and migration ability and to establish the role of these changes in treatment-associated EMD. Methods. We studied the effect of ATRA and IM on NB4 and K562 cells by combining adhesion/migration assays, microarray analysis, RT-PCR, Western blots and siRNA experiments. Results. 30-40% of ATRA-treated NB4 cells and IM-treated K562 cells adhered to fibronectin as opposed to untreated cells having no adhesion ability. NB4 cells adhered to FN even 5 days after VAD remission, whereas K562 adherence was found to be a 2.4-7.6-fold increase in the migration ability of NB4 and K562 cells following ATRA and IM treatment, respectively as compared to untreated cells. A microarray screen revealed alteration in the expression of many migration/adhesion related genes (pyk2, paxillin, integrin β2 and β7) following ATRA treatment. Following processing of the microarray results and relying on the decision to focus on the Pyk2 associated signaling complex, we continued our studies on 3 of the most relevant proteins in this complex: Pyk2, paxillin and integrin β2. We found that the mRNA and protein levels of these 3 key proteins are elevated following cellular exposure to ATRA. Moreover, Pyk2, paxillin and integrin β2 were found to be activated in response to ATRA treatment as seen by an increased phosphorylation levels or by the unveiling of activation-specific epitopes. Pyk2 activation is known to lead to the recruitment of paxillin and vinculin to Pyk2 located at focal adhesion sites. We observed an ATRA-dependent increase of Pyk2-paxillin and Pyk2-vinculin complex formation in our cells. In order to prove that Pyk2 is one of the key proteins regulating ATRA-induced cell migration and adhesion. Collectively our data support a critical role of Pyk2 in adhesion and migration initiated by various targeted therapies and a possible role in EMD development.

0384 CD38-SPECIFIC ANTIBODY DARATUMUMAB SYNERGIZES WITH NOVEL AGENTS LENALIDOMIDE AND/OR BORTEZOMIB TO IMPROVE THE ANTI-MYELOMA EFFECT EVEN IN LENALIDOMIDE/ BORTEZOMIB REFRACTORY PATIENTS

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Background. Multiple myeloma (MM) is a yet incurable malignancy of antibody-producing clonal plasma cells. Recently, significant progress has been made in MM treatment using novel immunomodulating agents such as lenalidomide (LEN) and bortezomib (BORT). Daratumumab (DARA) is a first-in-class human therapeutic CD38-specific antibody with broad-spectrum killing activity. Set out to further improve MM therapy by combining DARA with novel MM therapeutics, we have already shown the significant improvement of in vitro MM cell lysis by combining DARA with LEN. Methods. In ex vivo assays, which allow us to address killing of MM cells in bone marrow aspirates isolated from MM patients, we now explored the impact of combining DARA with LEN+BORT and with two recently introduced triple combination therapies: RDV (Len+Bort+Dexamethason) and MPV (Melphalan+ prednisone + BORT). Results. Addition of DARA to the combination of LEN and BORT significantly exceeded the effectiveness of the LEN-BORT treatment alone (p<0.001). Specifically, we observed a remarkable synergy between DARA and LEN/BORT in samples which responded poorly to LEN+BORT, including those samples obtained from 5 patients refractory to BORT and/or LEN. Furthermore, when combined with RVD and MPV, DARA almost doubled MM cell killing, especially in the low-dose range of the cocktails. Conclusion. Our results illustrate that treatment of MM with DARA in combination with novel multingdrug therapies bears great promise.

0385 ELIGLUSTAT, AN INVESTIGATIONAL ORAL THERAPY FOR GAUCHER DISEASE TYPE 1: PHASE 2 RESULTS AFTER 3 YEARS

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Background. Gaucher disease type 1 (GD1) is an inherited lysosomal storage disorder caused by deficient activity of acid β-glucosidase, a key enzyme in the degradation of sphingolipids. In GD1 patients, accumulation of glucosylceramide occurs primarily in tissue macrophages and leads to clinical manifestations, including thrombocytopenia, anemia, hepatosplenomegaly, and bone disease. Eliglustat, a novel ceramide analog that is a potent and specific inhibitor of glucosylceramide synthase, is under development as an oral substrate reduction therapy for GD1. Aim. To report long-term efficacy and safety results of eliglustat in GD1 patients. Methods. This ongoing, open-label, uncontrolled, multicenter, Phase 2 clinical trial of eliglustat (50 or 100 mg bid, depending on plasma trough levels) enrolled 26 previously untreated adults with GD1. Patients had to have splenomegaly with thrombocytopenia and/or anemia. The main efficacy outcomes included mean changes (± SD) from baseline in hemoglobin and platelet levels, spleen and liver volumes, and bone mineral density (BMD); the percentage of patients achieving therapeutic goals for anemia, thrombocytopenia, splenomegaly, and hepatomegaly (Pastores, Semin Hematol, 2004); and descriptive changes in skeletal lesions, infarcts, and femur dark marrow (reflecting marrow infiltration by Gaucher cells). Hematology, organ volumes, and biomarkers were assessed every 3-6 months. MRI, DXA, and X-rays were performed yearly and reviewed centrally. Results. Nineteen patients have completed 3 years of treatment, and 7 patients have discontinued the trial. After 3 years, hemoglobin increased by 2.6±1.39 g/dl (11.3±1.63 to 13.2±1.37 g/dl) and platelet count increased by 91±65.9% (70,000±21,700/mm3 to 126,800±40,500/mm3). Spleen volume (multiples of normal, MN) decreased by 61±12.2% and liver volume (MN) decreased by 29±15.8%. Most patients met long-term therapeutic goals for hemoglobin (100%), spleen volume (100%), liver volume (89%), and platelets (63%), and all patients met ≥5 therapeutic goals at 3 years. In 15 patients with evaluable DXA results at all timepoints, lumbar spine BMD increased by 0.6±0.69 Z-score and 0.6±0.9 T-score. Femur dark marrow was reduced (56%, 10/18) or stable (44%, 9/18) in 18 patients with findings at baseline. No bone crises or reductions in mobility occurred. There were no new lytic lesions, bone infarcts, fractures, or areas of osteonecrosis, and no worsening of pre-existing lytic lesions (8 patients) or bone infarcts (7 patients); 1 patient had worsening osteonecrosis noted retrospectively at baseline. Plasma GL-1 levels normalized, and median chitotriosidase and CCL-18 decreased by 80% and 73%, respectively. Eliglustat was well tolerated. Most adverse events (AEs) were mild (74%) and unrelated (95%) to treatment. The most common AEs were viral infections (6 patients); urinary and upper respiratory tract infections (4 patients each), and headache, increased blood pressure, abdominal pain, and diarrhea (3 patients each). Eight drug-related AEs, all mild, occurred in 6 patients. Summary/Conclusions. Eliglustat is a potential promising oral substrate reduction therapy for GD1. Clinically meaningful improvements have been observed in hematologic, visceral, and bone parameters, and most patients have met long-term therapeutic goals by 3 years. Eliglustat has been well tolerated and has led to the initiation of 3 international Phase 3 studies, which are actively enrolling patients.
Models of AML
Targeting PLK1 Activity with the Investigational Drug TAK960

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Background. Acute myeloid leukemia (AML) is one of the most prevalent forms of adult leukemia with very few long-term survivors. Novel approaches are urgently needed to improve clinical outcomes for patients. A better understanding of the factors that control AML pathogenesis will reveal new therapeutic targets and may offer an opportunity to improve patient survival. The siurni deacetylases (SIRTs) are a family of histone deacetylases (HDACs) with important roles in the regulation of genes that are essential for longevity, cell growth, tumor suppression, and apoptosis. Dysregulation of SIRT expression has been reported in several forms of cancer and could contribute to disease progression and drug resistance by increasing the lifespan and survival capacity of malignant cells. We hypothesize that SIRT1 is a critical regulator of the survival of AML cells and can be targeted for therapeutic benefit. Aims. (1) To elucidate the role SIRT1 in the growth and survival of AML cells. (2) To investigate the mechanism of action of the small molecule SIRT1 inhibitor tenovin-6 in preclinical models of AML. Methods. We tested our hypothesis in human AML cell lines, primary patient specimens and mouse models of AML. Results. We assayed the expression levels of SIRT1 and the related factor SIRT2 in normal mononuclear, a panel of human AML cell lines, and primary blasts patients with AML by quantitative RT-PCR and Western blot. SIRT1 was expressed at significantly higher levels in all AML cell lines and primary AML blasts compared with normal controls. In contrast, SIRT2 was expressed at very low levels in the majority of the samples analyzed. Although a number of HDAC inhibitors have been clinically investigated for cancer therapy, none of these drugs have significant inhibitory effects against SIRT1 or the related SIRT3. Therefore, the therapeutic potential of disrupting SIRT activity as an anticancer strategy remains to be rigorously investigated. Tenovin-6 is a novel small molecule inhibitor of SIRT1 activity. Treatment with tenovin-6 caused a dose-dependent reduction in AML cell viability and clonogenic survival and triggered apoptotic cell death. The tumor suppressor p53 is a SIRT1-regulated gene with a critical role in the cellular response to many classes of anticancer agents and its inactivation contributes to disease progression and drug resistance. Increased SIRT1 activity has been proposed as one mechanism by which cancer cells eliminate p53 function through chromatin silencing. Tenovin-6 treatment caused a dose-dependent accumulation of acetylated p53 in AML cells and increased expression of both p21 and PUMA. Targeted knockdown of PUMA with shRNA revealed that PUMA is a critical regulator of the pro-apoptotic effects of tenovin-6. Administration of tenovin-6 to mice was well-tolerated and led to a significant reduction in disease burden. Conclusions. SIRT1 is a very promising novel therapeutic target in AML. Further investigation aimed to elucidate the safety, efficacy, and mechanism of action of tenovin-6 is warranted.

Targeting PLK1 Activity with the Investigational Drug TAK960 Significantly Prolongs Survival in Preclinical Models of AML

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Background. Acute myeloid leukemia (AML) primarily affects elderly patients that have a reduced ability to tolerate intensive chemotherapy. Novel targeted agents offer an opportunity to achieve better therapeutic selectivity and improve clinical outcomes for patients with AML. Polo-like kinase-1 (PLK1) is a serine-threonine kinase that functions as an essential regulator of mitosis. Overexpression of PLK1 is a frequent event in cancer and is correlated with a poor prognosis. Its intrinsically pro-oncogenic properties in malignant cells and critical role in cell cycle regulation make PLK1 an attractive target for therapeutic inhibition. TAK960 is a novel small molecule inhibitor of PLK1 that has entered Phase I clinical trials. We hypothesized that inhibition of PLK1 would disrupt cell cycle progression, diminish AML cell viability, induce apoptosis, and antagonize in vivo leukemia pathogenesis. Aims. (1) To investigate the preclinical activity of TAK960 in preclinical models of AML. (2) To elucidate the mechanism of action of TAK960. Methods. The preclinical activity of TAK960 was investigated in human AML cell lines and disseminated and subcutaneous mouse models of AML. Results. Treatment with TAK960 disrupted the growth of 8 human AML cell lines. Acute exposure to TAK960 severely impaired the ability of AML cells to form colonies. Analysis of the effects of TAK960 on cell cycle progression demonstrated that TAK960 caused a substantial accumulation of cells with G2/M DNA content. TAK960 treatment led to the dose-dependent induction of apoptosis. Kinetic analyses revealed that cell cycle disruption occurred prior to the onset of apoptosis. We established a disseminated mouse xenograft model of AML with luciferase-expressing human AML cells to investigate the in vivo activity of TAK960. Administration of TAK960 to mice was well-tolerated, diminished the numbers of leukemic blasts in the bone marrow, and significantly prolonged animal survival. Analysis of specimens from mice treated with revealed a pharmacodynamic signature consistent with PLK1 inhibition. The in vivo efficacy of TAK960 was also evaluated in the subcutaneous MOLM-13 AML xenograft model. Administration of TAK960 significantly inhibited the growth of MOLM-13 tumors, disrupted cell proliferation and led to the induction of apoptosis. Clinical activity of TAK960 in AML. TAK960 is a novel PLK1 inhibitor that has potent activity in preclinical models of AML. Further investigation aimed to elucidate the safety, efficacy, and mechanism of action of TAK960 is warranted.

CARFILZOMIB-DEPENDENT SELECTIVE INHIBITION OF CHYMOTRYPSIN-LIKE ACTIVITY OF THE PROTEASOME LEADS TO IN VITRO AND IN VIVO ANTI-TUMOR EFFECT IN WALDENSTROM MACROGLOBULINEMIA

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Background. Selective inhibition of chymotrypsin-like (CT-L) activity of constitutive-(c20S) and immuno-(i20S) proteasome leads to a significant anti-neoplastic effect in a wide spectrum of hematologic tumors. Preclinical evaluation of new proteasome inhibitors with a more targeted inhibition of clonal cells is needed in order to increase efficacy and improve patient outcome. We evaluated the anti-tumor activity of Carfilzomib, a novel selective, irreversible peptide epoxyketone inhibitor of the CT-L activity of i20S and c20S, in WM, selective chymotrypsin-like (CT-L) and immunoproteasome (i20S) units, and they contain a higher i20S content as compared to normal BM, and in vivo. Aims. 1) To evaluate the distribution of i20S and c20S in WM primary cells as compared to the related normal cellular counterpart. 2) To evaluate the anti-tumor properties of Carfilzomib in WM, both in vitro and in vivo. Methods. Primary WM cells were obtained from bone marrow (BM) of WM patients (CD19+ microbead selection). WM and IgM secreting low-grade lymphoma cell lines were used. Level of immunoproteasomes (20S) and constitutive proteasome (20S) subunits were detected by an ELISA-based assay. Cytotoxicity, DNA synthesis were measured by MTT and thymidine uptake, respectively. Cell signaling and apoptotic pathways were determined by Western Blot. Effect of Carfilzomib on paracrine WM cell growth in the BM has been evaluated by looking at adhesion, migration and co-culture of WM cells with primary BM stromal cells (BMCs). Drug synergism was calculated using CalcuSyn software. In vivo studies were performed using B6C3Fl-GFP+/Luc+ cells injected into SCID mice, treated intra-venously with either Carfilzomib or vehicle. Detection of Carfilzomib-induced apoptosis has been validated ex vivo using WM cells isolated from BM of SCID mice treated with either vehicle or Carfilzomib. Measurement of human IgM has been performed on serum obtained from treated mice. Results. Primary WM cells which are characterized by higher expression of the i20S subunits as compared to c20S subunits, and they contain a higher i20S content as compared to normal CD19+ B-cells. Carfilzomib inhibited the CT-L activity of both i20S (LMP7) and c20S (β5) in primary WM cells, leading to inhibition of proliferation and induction of cytotoxicity; supported by increased PARP-, caspase-9-, -8 and -3-cleavage, as well as induced activation of c-jun-N-terminal kinase, and ER-stress in a dose-dependent manner. Carfilzomib targeted WM cells even in the context of BM milieu, where inhibition of adhesion and migration were observed, together with inhibition of WM growth even in presence of BMCs. Combination of carfilzomib and bortezomib induced synergistic cytotoxicity in WM
cells, as shown by enhanced PARP-, caspase-9- and -3-cleavage; and synergistic in inhibiting the CT-L activity of the i20S and c20S. Aneurin tumor activity of Carfilzomib has been validated in vivo, where carfilzomib-treated mice presented with a significant lower number of tumor cells (P<0.05); increased percentage of apoptotic WM cells (P<0.05); and reduced serum IgM levels (P<0.05), as compared to control mice. Summary. These findings demonstrate for the first time that Carfilzomib targets vaccinia in vivo, and in vivo, due to its anti-
CT-L activity of both i20S and c20S proteasome, providing the framework for testing this compound in this disease.

**0389** TREATMENT OF B-CELL MALIGNANCIES WITH EPRTUZUMAB ANTI-CD22-SN-38 CONJUGATES ALONE AND COMBINED WITH VELTUZUMAB ANTI-CD20 ANTIBODY THERAPY

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1 WITH VELTUZUMAB ANTI-CD20 ANTIBODY THERAPY
ANTIBODY THERAPY

Background. Many B-cell malignancies are responsive to antibody therapy, but more often, antibody therapy is combined with chemotherapy to optimize response. We developed procedures for coupling antibodies to SN-38, a highly potent topoisomerase I inhibitor that is the active component of the prodrug irinotecan. SN-38-conjugates of humanized antibodies epratuzumab anti-CD22 (Emab-SN-38) and veltuzumab anti-CD20 (Vmb-SN-38) were evaluated separately, and Emab-SN-38 was tested in combination with unconjugated Vmab. Methods. Emab and Vmab were conjugated with 6 moles of SN-38. The linker used in the preparation of these conjugates allows the SN-38 to be released slowly, with in vitro stability studies in human serum estimating that about 50% of the SN-38 is released within 1 to 1.5 days. With this type of linkage antibodies that are not internalized (i.e., Vmab), as well as internalizing antibodies (Emab), can be effective. In vitro and in vivo studies were performed to assess the activity of the conjugates against several B-cell lymphoma and leukemia cell lines. In vivo studies also examined combination therapy with Emab-SN-38 and unconjugated Vmab. Results. In vitro studies in 5 B-cell lymphoma cells lines (Daudi, Raji, Ramos, WSU-FSCCL, Jeko-1) and 4 acute lymphoblastic lymphoma cell lines (697, REH, MN-60, and RS4;11) expressing varying amounts of CD22 and CD20 determined by FACScan analysis, showed an IC50 ranging from 0.5 to 10 nM, confirming potent activity of each conjugate. Potency was not correlated to antigen content. Nude mice bearing SC Ramos human lymphoma had significant, yet similar anti-tumor activity, with both conjugates (0.25±0.01, 0.29±0.03 vs 0.20±0.01 grams; p=0.005, p=0.004). The number of regenerating myofibers was significantly increased by combined therapy with SN-38 and BM-derived EPCs (106±31.8), and in Shh-treated muscle (86.4±30.3) compared to untreated muscle (52.03±29.38) compared to control mice (30.5±8.8; p=0.00006, p=0.004). The number of regenerating myofibers was significantly higher in EPCs+Shh-treated mice (0.82±0.11) compared to control mice (0.70±0.04; p=0.01, p=ns). Capillary density was significantly higher in the in EPCs+Shh-treated muscle (106±31.8), and in Shh-treated muscle (86.4±30.3) compared to controls (62.2±17.7; p=0.0002, p=0.02). There was a significant increase in the muscle weight of mice treated with EPCs+Shh, and Shh versus controls (0.25±0.01, 0.29±0.03 vs 0.20±0.01 grams; p=0.005, p=0.004). The number of regenerating myofibers was significantly increased by the EPCs+Shh treatment (44.6±12.75), and in Shh-treated muscle (52.03±29.38) compared to controls (50.5±48.8; p=0.00006, p=0.004) (Figure 1).

**0390** COMBINED THERAPY WITH BONE MARROW-DERIVED ENDothelial PROGENITOR CELLS AND SONIC HEDGEHOG IMPROVES ANGIOGENESIS AND MYogenicITY IN AN EXPERIMENTAL MODEL OF MUsCULAR DYSTROPHY

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Recent work demonstrates that vascular abnormalities are required for organellar damage in Duchenne muscular dystrophy (DMD). Dystrophin is physiologically expressed in endothelial cells (ECs) and dystrophin-lacking ECs have significant impairment of flow-induced dilatation. It has been proposed that organ damage in DMD might be aggravated by such a defective arterial response to flow. These data suggest that increasing the vasculature in DMD may ameliorate the histological and functional phenotypes associated with this disease and indicate that, for an effective therapy of DMD, both the muscle and the vasculature need to be addressed. The bone marrow (BM) is an important source of ECs. In DMD, regenerative mechanisms, including angiogenesis, are constantly activated to contrast muscle degeneration, thus in this disease the contribution of BM-derived ECs to the process of regeneration might be substantial. We have recently demonstrated that Sonic hedgehog (Shh) gene therapy has angiogenic and myogenic potentials in the setting of ischemia. We investigated the hypothesis that combining Shh gene therapy with the administration of BM-derived endothelial progenitor cells (EPCs) increases angiogenesis and myogenesis in an experimental model of DMD. EPCs were obtained from the BM of 8-week-old C57BL/6J mice. We constructed a 4,878-bp plasmid containing the 600bp amino terminal domain coding sequence of human Shh. Unilateral hind-limb ischemia was created in 6 months-old mdx mice, the murine equivalent of DMD in humans. Each group was injected with either 1) PBS, 2) pShh (200 μg/mouse), or 3) pShh (200 μg/mouse) + EPCs (1×106 cells/mouse). Blood flow was measured in ischemic and contralateral hind-limbs by laser Doppler perfusion imaging at days 0, 7, 14, 21, and 28 after ischemia. At day 28, mice were sacrificed and aductor muscles were used to assess muscle weight, capillary density, and number of regenerating myofibers. At day 28 after ischemia, blood perfusion ratio between the ischemic and the contralateral leg was higher in EPCs+Shh-treated mice (0.86±0.07), and in Shh-treated mice (0.82±0.11) compared to control mice (0.70±0.04; p=0.01, p=ns). Capillary density was significantly higher in the in EPCs+Shh-treated muscle (106±31.8), and in Shh-treated muscle (91 d (2/10 surviving at 160 d), increasing to 91 d (2/10 surviving at 160 days) for unconjugated Vmab-treated animals. Emab-SN-38-treated animals had a median survival of 63d (0/10 surviving after 160 d), but when combined with Vmab, the median survival had not been reached at 160 d, with 6/10 surviving. Animals treated with a non-targeting IgG-SN-38 conjugate alone or combined with Vmab had a median survival of 65 d (0/10) and 91 d (2/10). The Emab-SN-38 conjugate combined with Vmab was significantly better than all treatment or control groups (P = 0.05). Similar enhancements were found in SC Ramos. Conclusion. Even at non-toxic dose levels, the Emab-SN-38 conjugate is a potent therapeutic, but responses could be enhanced significantly when combined with anti-CD20 immunotherapy. These data indicate Emab-SN-38 should be evaluated clinically alone and in combination with Vmab therapy.

In summary, Shh gene therapy had significant beneficial effects on blood perfusion ratio, capillary density, muscle weight, and number of regenerating myofibers, compared to PBS. These beneficial effects were further increased by combined therapy with Shh and BM-derived EPCs. Our results show that EPCs implantation combined with transfer of the human Shh gene improves angiogenesis in ischemic muscles of dystrophic mice. Further, this combined therapy enhances muscle mass and increases the number of regenerating myofibers in the mdx muscle. These findings represent a possible tool for future cell and gene therapy applications in DMD disease or other muscular dys dystrophies.
A TARGETED THERAPY (AVL-292) FOR BRUTON’S TYROSINE KINASE IN B-CELL MALIGNANCIES

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Background. Targeted therapies that suppress B cell receptor (BCR) signaling have emerged as promising agents in the treatment of B cell malignancies. Bruton’s tyrosine kinase (Btk) plays a crucial role in promoting B cell proliferation and survival through participation in the BCR signaling pathway and represents a promising new drug target.

Aims. Demonstrate the utility of a potent and selective inhibitor of Btk in B cell malignancies. Investigate effects of clinical candidate, AVL-292, on the survival and BCR activation of primary CLL cells cultured with Nurse-like cells (NLC). Determine, in a clinical setting, the minimum AVL-292 dose necessary for maximal Btk target site occupancy utilizing a novel translational medicine approach.

Methods. B lymphocyte cell signaling: Human naive, primary B cells or cultured Ramos human Burkitt’s lymphoma cells were incubated with compound for 1 hour followed by BCR stimulation with IgM. Antibodies used for immunoblot analysis include P-PLC-2, Btk and P-Btk. B Cell Proliferation: Human B cells were incubated with compound and anti-IgM for 56 h at 37°C and measured for [3H]-thymidine incorporation. Btk target occupancy: Cell lysates were incubated with the biotinylated covalent probe for one hour to detect free, unbound Btk. Lysates were then added to a streptavidin-coated plate to capture probe-bound Btk, followed by detection with an anti-Btk antibody. Results. AVL-292 is an orally active highly selective small molecule covalent Btk inhibitor that potently silences Btk enzymatic activity (IC50 < 0.5nM) and inhibits primary B cell proliferation and activation (EC50, 1-10nM). AVL-292 inhibits proliferation of multiple lymphoma cell lines which are dependent upon BCR signaling. AVL-292 also reduces survival and markers of BCR activation, such as CCL3 and CCL4 cytokine production, of primary CLL cells cultured with NLC. We have developed a covalent probe assay that enables direct measurement of Btk target site occupancy and can be used to correlate target site occupancy with activity of AVL-292 in vitro and in vivo.

Figure 1. Btk target occupancy in vivo.

RAPID TREATMENT OF SYMPTOMS OF TTP IN A BABOON MODEL

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Background. Thrombotic thrombocytopenic purpura (TTP) is characterized by hemolytic anemia and thrombocytopenia, with or without signs and symptoms of ischemic organ dysfunction. TTP has been associated with accumulation of ultra large von Willebrand Factor (ULVWF) due to deficiencies in the VWF cleaving protease ADAMTS13. These ULVWF multimers spontaneously interact with platelets in the microcirculation and promote the formation of platelet thrombi resulting in life-threatening microthrombosis. Recently, we have shown that all the clinical features of TTP can be induced in baboons by infusion of an inhibitory anti-ADAMTS13 antibody (SH9). TTP is currently treated by plasma exchange or plasma infusion. As spontaneous binding of platelets to the ULVWF molecules is responsible for the pathology, inhibiting the binding of platelets to ULVWF might be a good alternative treatment for TTP.

Aim. To test whether GBR600, an inhibitory anti-ULVWF-A1 domain monoclonal antibody previously shown to be safe and effective in preventing arterial thrombosis in a modified Folts model in baboons, will prevent and treat the symptoms of TTP in a baboon TTP model.

Methods. Eight baboons were given SH9 at 48 hour intervals to induce TTP for either 5 (n=3, prevention group) or 11 days (n=5, prevention group and control group). In the prevention group, baboons received in parallel a daily injection of GBR 600 (5 days) to see if this could prevent onset of TTP. In the treatment group (n=3), baboons received a daily injection of GBR 600 from the fifth day on to see if this would treat the symptoms of TTP. The baboons in the control (n=2) did not receive GBR600.

Results. Platelet count, haemoglobin concentration and % circulating schistocytes were monitored at regular intervals. As previously reported, injection of SH9 in baboons gradually induced thrombocytopenia reaching plateau counts of less than 30X10^9/l at day 4 in the control and treatment groups. Interestingly however, thrombocytopenia could be prevented when GBR600 was administered starting at day 1 of the SH9 injections (prevention group). The platelet count in the treatment group showed a steady increase from day 6 and returned to the baseline value in 72 hours after first injection of GBR 600. Haemoglobin concentration did not change significantly in the prevention group and decreased steadily in the control group for the duration of the study. In the treatment group there was a steady decrease in haemoglobin until day 6 after which it stabilized and started increasing from day 8. Prominent schistocytosis was seen in the control and treatment groups.

Summary. In this study we showed that inhibition of platelet binding to ULVWF can effectively prevent the onset of TTP as well as rapidly invert ongoing symptoms of TTP on the background of fully inhibited ADAMTS13.

THE INVESTIGATIONAL NOVEL MULTI-TARGETED AURORA B KINASE INHIBITOR TAK901 HAS POTENT ACTIVITY IN PRECLINICAL MODELS OF ACUTE MYELOID LEUKEMIA

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Background. The majority of patients diagnosed with acute myeloid leukemia (AML) are more than 60 years of age and their long-term prognosis is dismal. Pre-existing myelodysplasia, multidrug resistance, and con-existing morbidities limit therapeutic options for many patients. Novel approaches are urgently needed to improve clinical outcomes. The Aurora kinases (A, B, and C) are critical regulators of several events during mitosis. The overexpression of Aurora kinases in AML and co-existing morbidities limit therapeutic options for many patients. Novel approaches are urgently needed to improve clinical outcomes.

Aims. To test whether TAK901, a novel small molecule Aurora B kinase inhibitor with effects against a number of other...
kinases with important roles in cancer that is being evaluated in Phase I trials. We hypothesized that simultaneous targeting of Aurora B and other oncogenic kinases with TAK901 would disrupt cell cycle kinetics, inhibit proliferation, and induce AML cell death. Aims. (1) To determine the preclinical activity of TAK901 in AML. (2) To elucidate the mechanism of action of TAK901 in AML cells. Methods. Human AML cell lines and disseminated and subcutaneous mouse models of AML were utilized to investigate the preclinical activity of TAK901. Results. TAK901 potently diminished the growth and clonogenic survival of a panel of 8 AML cell lines. Treatment with TAK901 disrupted cell cycle kinetics leading to an accumulation of aneuploid cells, which occurred prior to the onset of apoptosis. A disseminated xenograft mouse model of AML was established using luciferase-expressing MV4-11 cell to investigate the in vivo anti-leukemic activity of TAK901. Administration of TAK901 to mice was well-tolerated and led to a highly significant increase in survival that was superior to what was achieved with the standard agent cytarabine. TAK901 induced AML cell apoptosis (active caspase-3) in vivo and dramatically diminished the phosphorylation of histone H3 in a manner consistent with Aurora B inhibition. Notably, TAK901 inhibited the infiltration of leukemic cells into the spleen and disrupted homing of AML cells to the bone marrow. The activity of TAK901 was also evaluated in the MOLM-13 subcutaneous AML xenograft model. Administration of TAK901 to mice bearing MOLM-13 tumors inhibited AML cell proliferation, induced apoptosis and led to disease regression. Conclusions. TAK901 is a novel multi-targeted Aurora B inhibitor that has preclinical activity in AML models and warrants further investigation.

0394
SAFETY AND TOXICITY OF INTRATHecal LIPOSOMAL CYTARABINE FOR TREATING CENTRAL NERVOUS SYSTEM (CNS) RELapse IN PEDIATRIC ACUTE LEUKEMIA: A MULTICENTER RETROSPECTIVE STUDY
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Background. The treatment of Central Nervous System (CNS) relapse in pediatric acute Leukemia (AL) remains a challenging clinical problem. Liposomal Cytarabine (DepoCyte) is a new intrathecal (IT) formulation characterized by the slow-release of free Cytarabine into the cerebrospinal fluid (CSF), resulting in longer drug exposure and a possibly higher leukemic response rate. Severe neurotoxicity has been reported with concomitant treatment. Therefore, we evaluated the safety profile of IT DepoCyte in a cohort of 26 pediatric AL patients with CNS relapse. Methods. Between May 2005 and August 2010 twenty-six patients (18 males, 8 females; 21 ALL and 5 AML; age at diagnosis 0.6-17 years; age at treatment 0.8-18 years) with CNS relapse were treated with IT DepoCyte at dosages ranging from 20 to 50 mg/dose depending on age, in seven Italian Pediatric Hematology Oncology Association (AIEOP) centers. Patients concomitantly received oral administration of Dexamethasone (DEXA) at a dosage of 0.2 mg/kg twice a day for 5 days, associated with IT DEXA in eleven children. Twenty three out of 26 patients were simultaneously treated with systemic chemotherapy. Concurrent high dose cytarabine or methotrexate was administered to 20 of these patients. DepoCyte was started upon CNS relapse and was administered every 15 days regardless of aplastic phase and underlying chemotherapy until CSF negativity in two consecutive lumbar punctures. Table 1 summarizes the clinical characteristics of patients. DepoCyte treatment was discontinued when neurotoxicity appeared or in the presence of severe adverse events. Toxicity was evaluated according to the National Cancer Institute Criteria. Results. 23 out of 26 patients (88.5%) achieved complete CSF remission and the remaining 3 presented partial remission (CSF negativity with persistence of neuroradiological findings of cerebral localization). The median number of administered doses was 4 (range 2-9), with CSF negativity after a median of three IT administrations. Neurological toxicity grade 3 was observed in 3 patients (11.5%); one patient experienced posterior reversible encephalopathy syndrome, the second had strabismus and clonus in the lower right limb and the third had partial seizures after CNS hemorrhagic stroke during the aplastic phase. Two of these 3 patients resumed DepoCyte after CNS event resolution without complications. We observed mild headache < grade 2 in 4 other patients. No permanent sequelae were observed. Two patients died of sepsis during treatment and 2 died from transplant-related complications; 8 died of non-CNS disease progression. Fourteen patients are currently in complete remission. Conclusions. The use of IT liposomal Cytarabine in the Italian experience showed acceptable tolerability and reasonable efficacy in the majority of patients. The frequency of neurological side effects was similar to what has been observed following other types of IT treatment. Despite its potential neurotoxicity, DepoCyte represents an interesting formulation since it reduces the frequency and total number of IT administrations. These characteristics associated with efficacy can improve compliance and quality of life in young patients. Further prospective studies on larger pediatric series are needed to confirm our observations and define optimal dosage and best timing administration of the drug in the pediatric setting.

0395
PRECLINICAL EVALUATION OF CPX-351 LIPOsome INJECTION IN A GENETICALLY ENGINEERED MOUSE (GEM) MODEL OF DRUG RESISTANT HUMAN ACUTE MYELOGENOUS LEUKEMIA (AML)
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Background. CPX-351 Liposome Injection is a nano-scale liposomal formulation that contains a synergistic 5:1 molar ratio of cytarabine (Cyt) and daunorubicin (Daun). Previous preclinical studies revealed that CPX-351 provides dramatic efficacy improvements compared to free drugs in a range of syngeneic and xenograft leukemia models.

Table 1.
Clinically, CPX-351 is being developed to replace conventional Cyt plus Daun therapy ("7+3"). A recent Phase II trial in newly diagnosed elderly AML patients treated with CPX-351 demonstrated improved outcomes over conventional "7+3" treatment with the largest improvements occurring in high-risk AML patients including CRs in patients that failed to respond to 7+3 prior to crossing over to CPX-351 treatment. Here we compare the therapeutic effects of CPX-351 and conventional Cyt:anthracycline in a GEM model exhibiting a genetic makeup common in refractory human AML to better understand the basis of the different responses observed between these two treatments. Aims. Assess the therapeutic activity of CPX-351 in a GEM leukemia model which reflects the genetics and pathology of refractory human AML. Methods. Luciferase expressing AML cells were implanted in C57Bl/6 mice, cells were prepared from AML mice induced by MLL/AP9 + Nras oncogene. Mice were monitored for bioluminescence signal every 4 days, starting 10 days after implant. Upon detection of a bioluminescent signal (pelvis, tail, femurs, hepatosplenic infiltration) treatments were initiated with CPX-351 (Q2D x3) or a cocktail of free Cyt (QD x5) and doxorubicin (QD x3). A no-treatment group served as control. Therapeutic activity was monitored by imaging before treatment, after last treatment, and 4 days after last treatment. Histopathological analysis of peripheral blood (May-Grunwald-Giesma) and mouse survival were also monitored. Results. Mice inoculated with refractory AML cells were treated once a bioluminescent signal indicative of leukemia cell engraftment was observed. Overall, more pronounced leukemia reduction (reduced bioluminescence) and survival was observed following CPX-351 treatment than was observed with free drug treatment. Images from mice that received no treatment displayed widely disseminated leukemia. Similar images of disseminated disease were observed in the free drug arm, however the extent and intensity required an additional 4 days to develop (8 days from treatment initiation) and only 5 mice survived. In contrast all 5 mice treated with CPX-351 were alive 8 days after treatment initiation and showed reduced bioluminescence than mice treated with free drugs. Peripheral blood smears obtained 3 days after treatment and on the day of euthanasia exhibited reduced leukemia cell burden. The resulting decrease in leukemia led to a statistically significant increase in 50% median survival when comparing the treatment arms. While the treatment-induced increase in lifespan (ILS) for the free drug cocktail was minimal (14%) the ILS for CPX-351 increased to 57%. Conclusions. CPX-351 was designed to enhance the efficacy of Cyt:Daun therapy by encapsulating both agents within a drug carrier that maintains the synergistic 5:1 molar ratio for extended times. Here preclinical data indicated that CPX-351 treatment of a model with a common genetic translocation for refractory human AML resulted in decreased leukemia burden and improved survival when compared to free drug treatment.

0396 ECULIZUMAB THERAPY FOR ATYPICAL HEMOLYTIC UREMIA SYNDROME IN PEDIATRIC PATIENTS: EFFICACY AND SAFETY OUTCOMES FROM A RETROSPECTIVE STUDY

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Background. Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease characterized by systemic thrombotic microangiopathy (TMA) due to chronic, uncontrolled terminal complement activation. Aims. To assess efficacy and safety of eculizumab treatment for aHUS outside of clinical trials. Methods. We conducted a retrospective data collection analysis of 30 aHUS patients receiving ≥1 eculizumab dose outside of clinical trials between 2007 and 2009. This report presents efficacy and safety outcomes for the 15 pediatric patients aged <12 years (<2 y [n=5]; 2-4 y [n=3]; 5-11 y [n=7]). Results. Baseline data and eculizumab efficacy outcomes for the 15 pediatric patients are presented (Table). Eculizumab efficacy was similar across the 3 age groups. Eculizumab safety in these pediatric patients was similar to eculizumab safety in adult and adolescent patients evaluated in clinical trials. Conclusions. In this medical practice setting, results for pediatric aHUS patients are consistent with results from adult and adolescent controlled trials in demonstrating that eculizumab treatment is well tolerated and can control TMA, improve kidney function and reduce need for plasma exchange/infusion, thus showing the promising potential of eculizumab as a new standard of care for aHUS.

Table 1.

Table 1.

FETAL HEMOGLOBIN INDUCTION BY A NEW HISTONE DEACETYLASE INHIBITOR

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Background. Induction of fetal hemoglobin (HbF) remains a promising therapeutic approach for the treatment of β-thalassemia and sickle cell disease, since HbF can substantially ameliorate the clinical symptoms of these genetic disorders. Several pharmacologic agents, such as hydroxyurea, 5-deoxyazacytidine, butyrate and trichostatin A have been shown to induce γ-globulin activity. However, the therapeutic use of these products is limited due to their relatively weak and variable efficacy, their cytotoxicity and possible long-term side effects. Thus, more effective agents that can induce higher HbF levels with lower toxicity are needed. One candidate group of substances with high therapeutic potential are the histone deacetylase inhibitors (HDIs), which through decondensation of chromatin structure can lead to reactivation of gene expression. Aims. To investigate the effects of a new histone deacetylase inhibitor (HDACi) on erythropoiesis and hemoglobin synthesis. Methods. An in vitro erythropoiesis model derived from human CD34+ progenitors cells from normal donors was used. HDACi effects on cell growth and viability, on erythroid differentiation and on HbF induction was investigated. Results. HDACi reduced cell growth and delay erythroid differentiation in a dose-dependent manner; concentration higher than 50nM significantly decreased the percentage of GPA+ and CD71+ cells and of mature orthochromatic erythroblasts. Lower concentration (1-50nM) didn’t affect cells proliferation and maturation (table 1). HDACi positively affected hemoglobin production, increasing the γ/β globin gene ratio and the HbF percentage. The increase observed with concent-
of the inducible shRNA system is warranted before in vivo experiments can be initiated. frida.ponthan@ncl.ac.uk

0399
TARGETING NAD+ SALVAGE PATHWAY IS A NOVEL THERAPEUTIC STRATEGY IN MULTIPLE MYELOMA
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Background. Nicotinamide adenine dinucleotide (NAD+) is a coenzymatic metabolite crucially involved in cellular transformation processes. During the neoplastic transformation, nicotinamide phosphoribosyltransferase (Namptase), a key enzyme involved in NAD+ biosynthesis, performs an important role since becomes upregulated to compensate for increased metabolic demands. Therefore, drugs capable to inhibit this enzyme, such as APO866, are under investigation for their potential as anticancer therapeutics. In this study we aimed to investigate the role of Nampt (the rate-limiting enzyme in the NAD+ salvage pathway) inhibitor APO866.

Methods. A panel of 14 MM cell lines, both sensitive as well as resistant to conventional chemotherapy, were used. Additionally 5 samples from peripheral blood of healthy donors were collected. 5x10^4 cells/well were plated in 96 well plates and treated with increasing concentrations of APO866 (range 10^-9-10^-6 M). Viability was assessed at 96 h from the beginning of the treatment by propidium iodide exclusion using a FACS CantoII.

Results. We found maximal cytotoxicity of APO866 against MM cell lines, with an IC50 values ranging from 3-30nM at 96h. Remarkably, in healthy leukocytes, APO866 was poorly active and failed to show any cytotoxic effect, indicating an increased reliance on these enzymes’ activity by MM cells. Titrated thymidine uptake assay confirmed the antiproliferative effects of APO866 in MM. Also the intracellular NAD+ levels lowered in the treatment with APO866 at 24 and 48 hours. A strongly expression of Nampt was revealed by western-blot analysis in all the cell lines analyzed. The AnnexinV/PI analysis confirmed APO866’s ability to induce apoptosis in a dose- and time-dependent fashion. Nampt inhibitor showed anti-myeloma activity even in the presence of interleukin-6 and insulin-like growth factor-1, confirming its ability to overcome the proliferative advantage conferred by this cytokines. Mechanistic studies, showed that APO866 cell death occurred in the context of Apoptosis, confirming its anti-myeloma activity.

Conclusion. Our preliminary data show the efficacy of Nampt inhibitor in MM cell lines, at nanomolar IC50 values ranging from 3-30nM at 96h. Remarkably, in healthy leukocytes, APO866 was poorly active and failed to show any cytotoxic effect, indicating an increased reliance on these enzymes’ activity by MM cells.

0400
PLUMBAGIN ENHANCES TRAIL-INDUCED APOPTOSIS OF HUMAN LEUKEMIC KASUMI-1 CELLS THROUGH UP-REGULATION OF DR5 EXPRESSION, ACTIVATION OF CASPASE-8 AND INHIBITION OF CFLIP
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Background. Although the patients with t(8;21) acute myeloid leukemia (AML) have a favorable prognosis when compared with other non-t(15;17) AML patients, only approximately 50% patients with this relatively favorable subtype are still alive at 5 years and refractory/relapse is still a tough problem in clinical. So the approach of finding new agents and/or methods is important. Aims: To investigate the effect of plumbagin alone, TRAIL alone and combination plumbagin/trail-induced apoptosis in leukemic Kasumi-1 cells and its mechanisms. Methods: Kasumi 1 cells were treated with plumbagin alone, rsTRAIL alone at different concentration, rsTRAIL combining with plumbagin. Cell proliferation was analyzed by CCK-8 assay.

0401
KNOCK-DOWN OF MLL/AF4 AND AML1/MTG8 AFFECTS PROLIFERATION AND DIFFERENTIATION OF AML AND ALL CELLS AND IMPAIRS CLONOGENICITY
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Background. MLL/AF4 and AML1/MTG8 are two fusion genes most frequently found in infant acute lymphoblastic leukaemia (ALL) and acute myeloid leukaemia (AML), respectively. We have previously shown that transient siRNA mediated knock-down of MLL/AF4 and AML1/MTG8 impairs proliferation and clonogenicity in vitro and causes a significant increase in median survival in a xeno-transplantation model. Aims. We aim to investigate the role of MLL/AF4 and AML1/MTG8 in leukaemic maintenance and progression of established disease in vivo. We used a constitutive and an inducible lentiviral shRNA expression system to determine the effects of knock-down of MLL/AF4 and AML1/MTG8 in the t(4;11)-positive SEM cell line and of AML1/MTG8 in the t(8;21)-positive human leukaemic cell line Kasumi-1. Methods. shRNA cassettes specifically targeting the MLL/AF4 and AML1/MTG8 genes were cloned into the pHR-eTAU vector, the lentiviral vector. SEM and Kasumi-1 cells were transduced with lentiviral particles produced in 293T cells, using lentiviral infection efficiency was determined by quantifying GFP or RFP positive cells, respectively, using flow cytometry. Knockdown expression of known targets of the fusion genes were verified at both the RNA and protein level by qPCR and western blotting respectively. Cell growth was monitored by cell counts and colony formation assays, including replating experiments were performed. Immunodominant NSG mice were transplanted by intrafemoral injection followed by monitoring of disease progression using bioluminescence. Results. More than 95% of SEM and Kasumi-1 cells were transduced with pHREshMLL/AF4 and pHREshAML1/MTG8 3 days after transduction but decreased to 90% after 30 days in culture. We confirmed that kasumi-1 cells transduced with pHREshAML1/MTG8 had decreased expression levels of AML1/MTG8 at protein and RNA levels associated with decreased expression of CD34, increased levels of IGFBP7 and an impaired clonogenicity. SEM cells transduced with pHREshMLL/AF4 showed decreased expression of MLL/AF4 with concomitantly decreased expression of HOXA7. The induction of shRNA expression systems showed similar results for both cell lines; however the transduction efficiencies were considerably lower compared to the constitutively expressed shRNA experiments. Currently, we are examining the in vivo consequences of fusion gene knockdown in our murine xenotransplantation model. Conclusions. shRNA knock-down of MLL/AF4 and AML1/MTG8 with either the constitutive or the inducible systems led to decreased proliferation and impaired clonogenicity and affected genes associated with differentiation. However the effects were delayed compared to transient siRNA knock-down. Further optimisation of
Apoptosis was studied independently through Annexin/PI double staining by flow cytometry and TUNEL staining. The expression of DR4 and DR5 at mRNA lever was detected by real-time PCR. The expression of signal transduction proteins, such as DR5, caspase-8, caspase-6, caspase-9, Bid, Bax and c-FLIP was detected by Western Blotting. caspase-8 and c-FLIP or plumbagin alone. TUNEL assay demonstrated that the number of apoptotic cells in groups of plumbagin combining with rsTRAIL were higher than the groups of rsTRAIL or plumbagin alone. Plumbagin could up-regulate the expression of DR5 at mRNA levels in Kasumi-1 cells by real-time PCR assay, and up-regulation of DR5, activation of caspase-8 and down-regulation of c-FLIP at protein level could be detected in plumbagin-alone and the combination with rsTRAIL groups. Conclusions: Plumbagin can enhance TRAIL induced apoptosis of Kasumi-1 cells, and the mechanism is in involving in the upregulation of DR5, activation of caspase-8 and the degradation of c-FLIP.

0402
A DRUG REPROFILING STRATEGY IDENTIFIES THE ANTI-HELMIANTIC NICLOSAMIDE AND VALPROATE (VAN) AS AN EFFECTIVE NOVEL ANTI-MYELOMA COMBINATION THERAPY THAT ALSO REDUCES FREE LIGHT CHAIN PRODUCTION

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Background and Aims. Our in vitro and in vivo studies have shown that rational drug redeployment (drug repurposing) can be used successfully to develop novel therapies for haematological tumours and bring significant clinical benefit. In this study, we have investigated a similar drug redeployment approach for multiple myeloma (MM). MM is a malignancy of differentiated B cells characterised by >10% neoplastic plasma cells in the bone marrow. In addition, monoclonal immunoglobulin (whole and/or free light chain) in serum/urine, lytic bone lesions and fractures, anaemia and immunodeficiency. Many patients also develop renal impairment (RI), predominantly caused by elevated nephrotoxic FLC secreted by the malignant plasma cell clone. Despite advances in therapy and improvement in survival times, particularly for younger patients, MM remains a fatal disease and a major cause of death. Furthermore, current intensive treatments are associated with significant toxicity and are not suitable for many MM patients especially the high proportion that are >70yrs. Hence novel therapies with low associated toxicities are urgently needed. The aim of this work was to identify drug combinations for MM using a screen of clinically available safe drugs. Methods. A panel of 100 off-patent oral drugs with low toxicity profiles, at drug concentrations consistent with peak serum concentrations achievable for routine clinical indications, were screened against MM cell lines. Cell numbers were assessed using Alamar Blue assays. Actuators were investigated using flow cytometry assays for apoptosis (annexin V/PI, Caspata, reactive oxygen species, cell cycle, and mitochondrial membrane depolarisation. Immunoblotting and/or immunofluorescence were used to measure LC3-II levels, and levels/localisation of components of NF-kB pathway. FLC protein was measured using luminex and flow cytometry and mRNA levels using quantitative real-time PCR. Results. The screen identified several drugs with anti-MM activity including valproate (already under investigation in myeloma) and niclosamide a broad spectrum anti-helminthic. The combination of niclosamide and valproate (VaN) exhibited greater anti-MM activity against cell lines and primary MM cells than either agent alone, and at least in vitro, out-performed the current anti-myeloma chemotherapy combinations of cyclophosphamide/thalidomide/dexamethasone (CTD) or melphalan/prednisolone (MP). Importantly, normal donor haematopoietic progenitor cells were significantly less sensitive to the treatments. Niclosamide treatment of myeloma cells was associated with the generation of mitochondrial superoxide (Mitosox) which was enhanced with the addition of valproate. Treatment with niclosamide was accompanied by rapid depolarisation of mitochondrial membranes as measured by TMRE and JC-1 staining. Cell death was associated with markers of apoptosis, annexin-V positivity and caspase activation. Individually, niclosamide induced markers of autophagy and valproate induced a G1 cell cycle arrest. Furthermore, sub-lethal doses of niclosamide reduced FLC protein secretion from MM cell lines, and some primary MMs. In some, but not all cases, reduced FLC protein secretion was associated with reduction in FLC mRNA transcription most likely through inhibition of NF-kB activity. Summary. This study identifies the potential of (DFO and DFX) (P<0.01). LIC and TIC in SIO were higher than in ICA treated groups. Survival was higher in SIO+DFX (P<0.01) but shorter in SIO+DFO (P<0.01). Conclusions. ICA may have concentration-dependent anti-tumor activity. In SIO with tumor, DFX may result in survival benefit and decreased iron content. We concluded that ICA might be a candidate for anti-tumor treatment along with chemotherapy in leukemia patients who have received multiple transfusions.

0401
IRON CHELATING AGENTS HAVE ANTI-LEUKEMIC EFFECT ON SECONDARY IRON OVERLOADED LEUKEMIA MOUSE MODEL

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Background. The patients with hematologic diseases receive multiple transfusions due to disease characteristics or treatments. In addition to the removal of iron, iron chelating agents (ICA) have several biological effects, including immunosuppression and changes in cell metabolism. Aims. We investigated the anti-tumor activity of ICA in an animal leukemia model with secondary iron overload (SIO). Methods. We used 5 week-old male BDF1 (H2b/d) mice, and leukemia or lymphoma cell lines A20, L1210, EL4 of B and T lymphoblast origin. The cell viability of cells was assessed in 48 hour culture under the various ICA concentrations with or without ferric chloride by CCK8 method. Also, we performed apoptosis analysis using flow cytometry. All mice were injected subcutaneously with L1210 cells in the right flank area, and measured for tumor mass and weight 3 times a week. All mice except those in the control group received iron dextran (10 mg/day) intraperitoneally for 20 days for SIO from day 7 of tumor injection. Deferoxamin (DFO) was injected intraperitoneally in dose of 40 mg/kg/day for 6 days, and deferasirox (DFX) orally administered at 20 mg/kg/day for 15 days from day 26 of tumor inoculation, respectively. We assessed tumor iron content (TIC), liver iron content (LIC), tumor size, and survival. Results. The viability of A20 and L1210 decreased more than that of EL4 under the ICA. The viability of L1210 under DFX decreased more than that under DFO in therapeutic concentrations (P<0.01). The percentage of apoptosis was dependent on the concentration of DFO and DFX, although there was more apoptosis in DFX treated group than in DFO group (P<0.01). The expressions of Fas on L1210 did not change according to ICA concentration. Sizes of tumor mass between groups were not different until ICA administration (P>0.05). However, tumor grew rapidly in untreated groups (control and SIO) but slowly in ICA treated groups (DFO and DFX) (P<0.01). LIC and TIC in SIO were higher than in ICA treated groups. Survival was higher in SIO+DFX (P<0.01) but shorter in SIO+DFO (P<0.01). Conclusion. ICA may have concentration-dependent anti-tumor activity. In SIO with tumor, DFX may result in survival benefit and decreased iron content. We concluded that ICA might be a candidate for anti-tumor treatment along with chemotherapy in leukemia patients who have received multiple transfusions.

FREE LIGHT CHAIN PRODUCTION ANTI-MYELOMA COMBINATION THERAPY THAT ALSO REDUCES NICLOSAMIDE AND VALPROATE (VAN) AS AN EFFECTIVE NOVEL ANTI-MYELOMA COMBINATION THERAPY THAT ALSO REDUCES FREE LIGHT CHAIN PRODUCTION
INHIBITION OF MTOR WITH EVEROLIMUS (RAD001) AND SILENCING BY VASCULAR ENDOTHELIAL CELL GROWTH FACTOR SPECIFIC siRNA INDUCES ADDITIVE ANTITUMOR ACTIVITY IN MULTIPLE MYELOMA CELLS

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Background. Angiogenesis plays an important role in the progression of solid tumors. Targeting angiogenesis can be a valid therapeutic approach. We have demonstrated the anti-tumor activity of everolimus (RAD001), an mTOR inhibitor, in a variety of cancers, including various myeloma cell lines. Several RNA interference (RNAi) methods are rapidly being established and hold promise to specifically inhibit gene expression in mammals. RNAi is the sequence-specific, posttranscription gene silencing methods initiated by double-stranded RNAs, which are homologous to the gene being suppressed. We have previously reported that Zn and colchicine are effective, affordable, non-myeloablative therapies. The study was to identify novel treatments for BL by screening off-patent, non-toxic, non-myeloablative, oral drugs for anti-BL activity.

Aim. These findings support the ongoing phase I clinical trial of PDX/BEX in sub-Saharan Africa; Identification of Zn and colchicine (ZaC) as potential affordable non-toxic therapy for endemic Burkitt’s lymphoma

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Background. Burkitt’s lymphoma (BL) is a high-grade B-cell Non-Hodgkin’s Lymphoma (NHL) with one of the fastest doubling time amongst human tumours. Occurring most frequently in children in areas with holoendemic and hyperendemic malaria, endemic BL (eBL) accounts for ~50% of all childhood malignancies in sub-Saharan Africa. Although intensive chemotherapy is highly effective against BL with potential cure rates of ≥85% the financial implications of high-intensity regimens including the cost of drugs, transfusions, and intensive supportive medical care preclude their use in many developing countries for the majority of patients. Low-dose chemotherapy combinations based on cyclophosphamide cost <US$50 per patient have managed to achieve ~50% 1year disease free survival in eBL (95% chance of a cure). However there is a desperate need for effective, affordable, non-myeloablative therapies. The aim of this study was to identify novel treatments for BL by screening off-patent, non-toxic, non-myeloablative, oral drugs for anti-BL activity. Methods. A panel of 100 off-patent drugs were investigated using flow cytometry based assays for apoptosis (annexin V/PI, Caspateg), reactive oxygen species (ROS) and cell cycle. Normal donor bone marrow samples were treated and viability assessed using immunophenotyping and flow cytometry. Results. The drug screen performed using peak serum drug concentrations identified colchicine and ZaC as having potential anti-BL activity, indicating that ZaC therapy is unlikely to be myeloablative. Conclusions. These findings suggest that MTOR inhibition and silencing by VEGF specific siRNA may be associated with an additive antitumor activity and might be a suitable target for new therapeutic strategies using RNA interference in MM.

PRLATREXATE SELECTIVELY INDUCES APOPTOSIS AND SYNERGIZES WITH BEXAROTENE THROUGH UP-REGULATION OF P53/BAX/PUMA IN CUTANEOUS T-CELL LYMPHOMA CELLS

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Background. Prlatrexate (PDX), a targeted antifolate, was designed for preferential uptake and accumulation in tumor cells based on its high affinity for reduced folate carrier-1 and efficient polyglutamation by folypolyglutamyl synthetase. PDX is approved by the United States FDA for relapsed or refractory peripheral T-cell lymphoma, and has demonstrated activity in a Phase I study in cutaneous T-cell lymphoma (CTCL). Bexarotene (BEX), an all-trans-retinoic acid (ATRA), is an RXR-selective retinoid, and thus of interest to study in combination with PDX. Aims. In this study, we investigated the therapeutic mechanisms of PDX and whether combination with BEX has synergistic anti-tumor effects in CTCL. Methods. Cell viability was examined by MTS assay and apoptosis by FACS analysis. Results. The combination of PDX/BEX synergistically increased the tumor suppressor p53, and the apoptosis effect through up-regulation of p53-regulated pro-apoptosis proteins, Bax, and PUMA. Conclusions. These experiments highlighted additive effects of the combination (zinc and colchicine) as observed in some of the lines. These data indicate that ZaC is an effective, affordable, therapeutic option for endemic BL.
0406
DNA-DEPENDENT PROTEIN KINASE AS A PROMISING MOLECULAR TARGET FOR THE TREATMENT OF ADULT T-CELL LEUKEMIA-LYMPHOMA

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The DNA repair system is a promising target for sensitization and overcoming drug resistance on cancer treatment. Since the cells that genetically too unstable will die, a treatment that blocks a particular DNA repair system can induce apoptotic cell death on cancer cells but not on normal cells. We recently found that high expression of catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) was observed in adult T-cell leukemia-lymphoma (ATL) cells. In addition, a new chemotherapeutic drug NK314 possessing inhibitory activity for both topoisomerase II and DNA-PK potently inhibited the growth of various ATL cell lines. According to the results, we designed combination treatment with various DNA-PK inhibitors and chemotherapeutic agents. NU7026, a DNA-PK inhibitor, enhanced the anti-cancer activity of etoposide. Such enhancement of cell growth inhibition by DNA-PK inhibitors was not observed in DNA-PK deficient cancer cell line, M059J cells. These results suggested that DNA-PK is a promising target molecule for ATL. We also identified that hnRNP B1, a RNA binding protein, directly bound with DNA-PK complex and inhibited DNA-PK activity in lymphoid malignant cells. Since hnRNP B1 is overexpressed in a population of ATL cells, hnRNP B1 can be a prediction marker for response to combination therapy with DNA-PK inhibitors and chemoradiotherapy.

Red blood cells and iron - Biology

0407
ENUCLEATION OF HUMAN ERYTHROBLASTS IS AN ACTOMYOSIN-DEPENDENT PROCESS FOLLOWING NUCLEAR POLARIZATION

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Backgrounds. During erythropoiesis, stem cells undergo lineage specific commitment and generate erythroid progenitor cells through cellular division events including nuclear (mitosis) and cytoplasmic (cytokinesis) division. In terminally differentiated erythroblasts, the centrally located nucleus becomes ecentrally located nucleus via a process known as polarization, and is expelled via a process termed enucleation, becoming reticulocytes and subsequently mature erythrocytes. Enucleation of erythroblasts is thought to occur through a process similar to cytokinesis. However, little is known in regards to how the process of enucleation differs from conventional cytokinetic processes as well as to the precise role of non-muscle myosin II in enucleation. Aim. The aim of this study is to investigate how the process of enucleation differs from conventional cytokinetic processes, and to elucidate the role of cytoskeletal modifiers during each step of enucleation in human erythroblasts. Methods. We investigated the role of cytoskeletal modifier during conventional erythroid cell division of early-stage erythroid cells such as colony-forming unit-erythroid (CFU-E), and compared their roles with that during each step of enucleation in human erythroblasts. For this purpose, highly purified human CD34+ cells were induced to differentiate to the level of CFU-E, and to the level of terminally differentiated erythroblasts that undergo enucleation. Since the efficacy of inhibitors for cell division often varies depending on the species of the cell observed, the immortality of the cells and their redundancy in the cells themselves, efficient inhibitors of cell division in human CFU-E were selected, and then examined the effects of these inhibitors on nuclear polarization and enucleation events. Results. We selected blebbistatin, an inhibitor of non-muscle myosin II ATPase, cytokalasin D, an inhibitor of actin polymerization, NSC23766, an inhibitor of Rac 1 GTPases, Y27632, an inhibitor of ROCK, colchicine and vinblastine, inhibitors for microtubules, and monastrol, an inhibitor of the mitotic kinesin Eg5, as efficient inhibitors for CFU-E cell division. When these inhibitors were applied to terminally differentiated erythroblasts, blebbistatin, cytochalasin D increased the number of cells with a polarized nucleus accompanied with a complete block of enucleation. NSC23766 and Y27636 appeared to increase cells with nuclei positioned in the center of the cytoplasm, and caused an immediate and complete inhibition of enucleation. Colchicine, vinblastin and monastrol did not increase cells with a polarized nucleus and did not inhibit enucleation. The degree of the increase in reticulocytes during the initial 24 h was similar to that of cells with a polarized nucleus. Conclusion. This study shows that the inhibition of non-muscle myosin II ATPase and ROCK completely blocked enucleation of human erythroblasts, demonstrating for the first time that non-muscle myosin II is required for human erythroblast enucleation. We also suggest that tubulin, kinesin, Rac GTPases and ROCK may be involved in nuclear polarization that is required just prior to enucleation. It is anticipated that these advances will enable the definition of defects in enucleation of erythroblasts in inherited and acquired red cell disorders, and in bone marrow failure syndromes.

0408
GENE EXPRESSION PROFILE ANALYSIS IN HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN (BRAZILIAN TYPE): IDENTIFICATION OF TARGET GENES THAT COULD BE RELATED TO HEMOGLOBIN SWITCHING

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Hereditary Persistence of Fetal Hemoglobin (HPFH) is a heterogeneous and benign group of genetic disorders characterized by an abnormal switching from fetal to adult hemoglobin, resulting in increased fetal hemoglobin (HbF) levels at the adult stage. The reactivation of HbF...
is an important therapeutic option in patients with hemoglobin disorders, since it inhibits the polymerization of sickle hemoglobin. The non-deletional Brazilian type HFPH (BHPFH), a C[ARROWRIGHT]G mutation at 195 position of the A gamma globin gene, was first identified in 1990, but the underlying mechanism of upregulation of fetal hemoglobin is still unclear. In order to elucidate how this point mutation leads to an altered gene expression pattern, the aim of this study was to identify gene expression patterns by generating novel cDNA libraries from peripheral blood samples isolated from patients presenting with high levels of fetal hemoglobin. These patients were treated with hydroxyurea and the expression of tissue factor (TF), a marker of coagulation activation, was assessed. Our study showed the presence of mutations within the KLF1 gene, leading to high levels of HbF in adults. Elevated HbF levels may ameliorate the clinical phenotype of beta-thalassemia. Aims. The aim of this study was to investigate the possible correlation between mutations within the KLF1 gene and its expression levels in patients with high levels of HbF, who were shown to be negative for mutations in the γ-globin genes. Methods. Mutation screening in the KLF1, HBF, HBG1 and HBG2 genes was done using PCR and direct sequencing. Results. Three adult patients presented with high levels of HbF (11-17.7%) and without mutations in the HBF, HBG1 and HBG2 genes, were analyzed for the presence of mutations in the KLF1 gene. Sequencing analysis revealed the presence of two mutations, namely p.S102P and p.F182L, within exon 2 of the KLF1 gene. One patient (HbF=12%), homozygous for the p.S102P mutation, was shown to be heterozygous for p.F182L, within exon 2 of the KLF1 gene. Other two patients (HbF11% and HbF17.7%) were heterozygous for p.S102P and p.F182L, respectively. Conclusion. Our study showed the presence of mutations within the KLF1 gene in patients with elevated HbF levels. We propose that these mutations, in single or compound heterozygosity, influence KLF1 and BCL11A gene expression, which, in turn, affects transcription of the γ-globin genes and, hence, HbF production.

**0410**

**KLF1 GENE MUTATIONS ARE ASSOCIATED WITH HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN AND DECREASED KLF1 AND BCL11A GENE EXPRESSION LEVELS**

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**Background.** Hereditary persistence of fetal hemoglobin (HPFH) is characterized by persistent high levels of fetal hemoglobin (HbF) in adults. Several genetic factors that control HbF levels in adults have already been identified (HBB, HBS1L-MYB, BCL11A genes), while others remain elusive. Recent studies have reported mutations within the KLF1 gene, leading to high levels of HbF in adults. Elevated HbF levels may ameliorate the clinical phenotype of beta-thalassemia. Aims. The aim of this study was to investigate the possible correlation between mutations within the KLF1 gene and its expression levels in patients with high levels of HbF, who were shown to be negative for mutations in the γ-globin genes. Methods. Mutation screening in the KLF1, HBF, HBG1 and HBG2 genes was done using PCR and direct sequencing. Results. KLF1 and BCL11A gene expression levels were quantified by Q-PCR. Results. Three adult patients presented with high levels of HbF (11-17.7%) and without mutations in the HBF, HBG1 and HBG2 genes, were analyzed for the presence of mutations in the KLF1 gene. Sequencing analysis revealed the presence of two mutations, namely p.S102P and p.F182L, within exon 2 of the KLF1 gene. One patient (HbF=12%), homozygous for the p.S102P mutation, was shown to be heterozygous for p.F182L, within exon 2 of the KLF1 gene. Other two patients (HbF11% and HbF17.7%) were heterozygous for p.S102P and p.F182L mutation only. However, in healthy, serotonin controls none of these mutations were detected. Q-PCR analysis in the patient, homozygous for p.S102P mutation and heterozygous for p.F182L mutation, showed that KLF1 gene expression levels were more than 60% lower than in healthy controls, while BCL11A gene expression levels were more than 45% lower than in healthy controls. Conclusion. Our study showed the presence of mutations within the KLF1 gene in patients with elevated HbF levels. We propose that these mutations, in single or compound heterozygosity, influence KLF1 and BCL11A gene expression, which, in turn, affects transcription of the γ-globin genes and, hence, HbF production.

**0411**

**VARIANTS IN GENETIC MODIFIERS OF BETA-THALASSEMAIA CAN HELP TO PREDICT CLINICAL SEVERITY**

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**Background.** Patients with beta-thalassemia display large variability in disease severity and are usually classified into thalassemia major...
(TM) or intermedia (TI) according to clinical criteria. The major determinant of the severity is the degree of beta-globin chain deficit resulting from the nature of the beta-thalassemia mutations. Other genetic modifiers, affecting the degree of alpha and non-alpha-globin chain imbalance, also impact the phenotypic severity: an associated alpha-thalassemia minimizes the excess of alpha-globin chains and tends to produce a less severe condition. An increased residual level of HbF in adult life is also a major amelioration determinant. Three major HbF Quantitative Trait Loci (QTL) have been identified so far. The so-called -158 C>T XmnI SNP, is located in the fetal Ggamma-globin gene promoter. The two others are located in the BCL11A and in the HBSB1-cMYB inter-region. Some particular tag-SNPs in these regions are associated with high HbF levels in healthy adults and in Thalassemia patients. Aims. In this study, we investigated the effect that these SNPs might exert in combination with beta and alpha-thalassemia genotypes on beta-thalassemia severity. Method. A cohort of 101 affected patients, included in the French National Registry for Thalassemia, were classified into TM (n=69) or TI (n=32) according to clinical data. All patients were genotyped for (i) beta-thalassemia mutations, (ii) the XmnI SNP, (iii) the -3.7 kb alpha-thal deletion, (iv) the tag-SNP rs11886868 in BCL11A exon 2 and (v) the tag-SNP rs93991917 in the HBSB1-cMYB inter-region. Univariate and multivariate analyses were performed to study the risk of TI associated with the presence of favourable alleles. Results. As expected, univariate analysis showed that beta-thalassemia mutations and XmnI -158 C>T SNP have the strongest effect on severity. Multivariate analysis performed with the 5 modifiers indicated that presence or absence of these favourable alleles could predict the type of Thalassemia in 85.8% of the cases (major type: 93.2%, intermediate type: 69%). The predictions made from the beta-thalassemia mutations and the XmnI SNP alone were significantly improved by the adjustment with the 3 other modifiers, moving from 75.2% to 83.8% (p<0.001). In order to test an easy-to-use prediction tool, we calculated a ‘score variable’ defined as the number of favourable alleles carried by each patient. Following this simple scoring, all patients with score 0 were TI (96% with score 0 or 1) whereas all patients with score 5 or 6 were TI. When considering only the beta0/beta0 patients, the scores ranged between 0 and 5 and became informative for all patients: more than 95% patients with a score between 0 and 2 were TI; more than 95% patients with a score between 3 and 5 were TI. Conclusion. In this study, we showed that predictions based on genetic modifiers can predict the major or intermediate type of beta-thalassemia, even in cohorts of patients with various beta-globin genotypes (up to 30 different mutations in our series). If further validated, this prediction tool of severity may have implications for genetic counselling but also for decisions regarding therapeutic options such as HSC transplantation.

0412 TELOMERASE ACTIVITY IS USEFUL FOR THE SCREENING OF CRYPTIC AND LATE ONSET DYSKERATOSIS CONGENITA AND THE EVALUATION OF THE TREATMENT RESPONSE TO ANABOLIC STEROIDS FOR THEIR BONE MARROW FAILURE

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Background and Aims. We have recently shown that some patients with cryptic and late onset Dyskeratosis Congenita (DKC) among those with acquired aplastic anemia (AA) or myelodysplasia syndrome have short telomeres due to mutation in the components of telomere-associated genes such as TERC, TERT or TINF2. Clinically it is very important to identify cryptic and late onset DKC patients because these patients will exhibit refractoriness to conventional immunosuppressive therapy (IST) for their bone marrow failure. Several groups, including our own (15th. Congress of European Hematology Association, 2009, Barcelona, Spain), revealed telomere length in peripheral blood was useful in the screening of these patients, but some patients with telomere-associated gene mutation did not have short telomere. The reasons of normal telomere length in these patients might be that aging and accelerated generation were insufficient. Moreover, recent study reported that the androgen, sex steroid hormone, induced hematopoiesis by activating telomerase in hematopoietic cells. In this study, we studied telomerase activity in peripheral blood from patients with DKC and AA patients to clarify its usefulness in diagnosis and the evaluation of the treatment response to anabolic steroid. Methods. We analyzed telomerase activity in peripheral blood from one DKC, three cryptic DKCs, 15 AA patients, and 30 healthy controls. Telomerase activity of the cellular extract from 2x10^5 peripheral mononuclear cells was assayed using the TRAPEze Telomerase Detection Kit. We analyzed the entire coding region of each telomere-associated genes by direct sequence, and measured the length of telomeres in peripheral blood by Southern blot analysis. Results. Telomerase activity of DNA isolated from unaffected blood cells was significantly higher than those of healthy controls (16.3 TPG unite vs 86 TPG unite, p=0.011). Telomerase activity did not significantly differ between AA patients and healthy controls (77.3 TPG unite vs 86 TPG unite, p=0.325). However we identified two AA patients who showed obviously lower telomerase activity (53 and 44 TPG unite) than healthy controls. One of these AA patients showed hemoglobin (Hb) level lower than 5 g/dL with mutations of telomere associated genes. These AA patients with lower telomerase activity did not responded to IST, and may have unknown mutations of telomere associated genes. Next, we analyzed the relationship between telomerase activity and the clinical responses to anabolic steroid in one DKC, two cryptic DKGs, and 3 AA patients who were refractory to IST. The anabolic steroid significantly increased telomerase activity in DKC patient (from 15.7 to 54 TPG unite, p=0.002) and tended to increase telomerase activity in cryptic DKC patients (from 21 to 42 TPG unite, p=0.061) and AA patients (from 77 to 98 TPG unite, p=0.052). Clinically, a slight increase of hemoglobin (from 9.8 to 10.9 g/dL) was observed in DKC patient, but no obvious increase in hemoglobin was seen in other patients. Conclusions. These findings revealed that the assay of telomerase activity is useful for screening cryptic DKC, but that longer follow-up may be necessary to evaluate clinical response to anabolic steroid.

0413 GROWTH DIFFERENTIATION FACTOR 15 (GDF-15) PRODUCTION IN COBALAMIN DEFICIENCY ANEMIA

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Background. Cobalamin (or vitamin B12) is important to the normal production of thymidine, and its deficiency may prevent normal DNA synthesis in bone marrow precursors causing megaloblastic anemia. Ineffective erythropoiesis is a hallmark of this condition and high levels of lactate dehydrogenase (LDH) with mild unconjugated hyperbilirubinemia due to intramedullary hemolysis have been described. Inherited sideroblastic anemias, including DKC, are also present with ineffective erythropoiesis, and recent studies have shown that erythroblasts in the ineffective bone marrow produce large amounts of Growth Differentiation Factor 15 (GDF-15), a potent downregulator of hepcidin, resulting in increased iron uptake and consequent iron overload. Aims. To determine GDF-15 plasmatic levels in patients with megaloblastic anemia, and correlate with iron homeostasis and hematological parameters during the normalization of erythropoiesis after cobalamin replenishment. Methods. We selected patients with proven cobalamin deficiency anemia, as confirmed by a typical blood smear (macro-ovalocytes, neutrophil hypersegmentation), low serum cobalamin (<200pg/ml) along with normal serum ferritin levels and characteristic clinical presentation (anemia with or without neurological symptoms). Peripheral blood samples were collected upon informed consent at diagnosis, 7 days after daily 5mg intramuscular cyanocobalamin treatment and after complete normalization of hematological parameters. Ferritin, transferrin saturation and LDH levels were all late determinations performed by routine laboratory methods. GDF-15 plasmatic levels were determined by ELISA assay. Results. Fourteen patients were enrolled with symptomatic cobalamin deficiency between August 2009 and September 2010 (10 male/4 female, age range 28-80 years old). Results are expressed as mean±SEM. Mean cobalamin levels (472±107pg/ml) and transferrin levels increased from 15.6±5.1 to 29.3±6.8 mg/dL (p=0.0005) and transferrin saturation were in the normal range, 227.2±41.4 ng/mL and 32.9±5.0%, respectively. As expected, mean hemoglobin (Hb) levels increased with treatment (8.75±0.41 g/dL at diagnosis (D0), 10.64±0.54 g/dL after 7 days of treatment (D7) and 13.02±0.45 g/dL after normalization (D17), p<0.0001). Mean erythrocyte count decreased with treatment (11.4±2.5 fl (D0), 110±2.6 fl (D7) and 91±4.2fl (D17), p=0.0005). GDF-15 levels were 8057±2215pg/mL at D0 (range 1933–171

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significant correlation between GDF-15 levels and Hb levels (r²=0.34, p<0.0001), but not between GDF-15 and LDH levels (p=0.45). Conclusions: Treatment with cyanocobalamin restored normal erythropoiesis while decreasing GDF-15 levels to baseline levels before normalization of hematological parameters. The lack of correlation with LDH levels, unspecifically produced in increased cell proliferation, is consistent with reports that only immature erythroblasts produce large amounts of LDH. While increased LDH overproduction of GDF-15 has been reported in anemia of chronic disease not primarily affecting iron homeostasis, and erythropoiesis-driven high GDF-15 levels in thalassemia have been linked to iron overload, there was no association between ineffective erythropoiesis and spontaneous iron overloading of the thalassemic bone marrow. This suggests that long periods of time exposure to increased GDF-15 levels may be needed for iron overload to take place in this setting. The pathophysiological role of the iron regulatory effect of GDF-15 in megaloblastic anemias should be further investigated.

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0414 ALTERATIONS IN CELL TURNOVER AND PRO-APOTOTIC SERUM FACTORS MAY MODULATE NEUTROPHIL NUMBERS IN SICKLE CELL DISEASE
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Background. Leukocytes are known to exacerbate inflammatory and vaso-occlusive processes in sickle cell disease (SCD) by adhering to the vascular endothelium and participating in inflammatory mechanisms. The relevance of neutrophil death to inflammatory disease pathogenesis is recognized, since alterations in leukocyte apoptotic processes may affect cell function and inflammatory processes. Aim. Since previous data indicate an inhibition of neutrophil apoptotic pathways in SCD, the present study investigated whether alterations in SCD neutrophil (SCDneu) death pathways are the result of a shift in cell turnover and/or whether factors present in SCD serum (ser) influence this process. Methods. Two different cultures from human K562 cells (1x10^6 cells/mL in DMEM, 10% FBS, penicillin/streptomycin, 5% CO2, 37°C) were transfected with control or EYA3 knockdown lentivirus (MOI=1.0). After proliferation and selection of positive cells with puromycin (2.0 µg/mL), cells were treated with 30µM hemin and collected after 0, 24, 48, 72 and 96h for gene expression and flow cytometry analysis. Table S1: hematopoietic cells from 4 control individuals were proliferated and differentiated into late stage erythroblasts. EYA3, α, β and γ-globin gene expression was analyzed by qRT-PCR and quantified using the Gnorm program. HbF expression and apoptosis were analyzed by flow cytometry.

Results. Analysis of α, β and γ-globin expression shows that these genes are downregulated in K562 culture cells knockdowned for EYA3 compared with a control culture at 0, 24, 48, 72 and 96h after hemin addition (see Table 1). Hemin-induced EYA3 knockdown in SCDneu induced a significant increase in cell death (24h: 5.3±1.3%; P<0.0001; 48h: 6.4±1.4%; P<0.0001; 72h: 7.5±1.7% PI positive cells; n=7; P<0.01). Whilst SCDneu cultured in the presence of CONser were higher (24h: 4.4±1.4%; P<0.0001; 48h: 5.4±1.2% PI positive cells; n=7; P<0.01), unexpectedly, the culture of CONneu incubated with SCDser was significantly augmented apoptosis (56.5±5.4%; n=8) compared to CONneu cultured with CONser (44.4±4.2%; P<0.0001; n=8) or SCDHser (45.1±4.8%; P<0.0001; n=8; compared to SCD; Annexin V binding). Caspase-9 activity was also significantly increased when CONneu were cultured for 16h in SCDser, compared to CONser (0.025±0.003; 0.016±0.002 OD; n=12; P=0.01, respectively). However, caspase-9 gene expression was not different between the groups (data not shown). Incubation of neutrophils with the presence of the programmed necrosis inhibitor, Necrostatin (10µM), reduced death cell independently of serum type (2.2±0.3; 0.6±0.06% propidium iodide staining; P<0.001 for CONser; 2.9±0.5; 1.1±0.2%; P<0.001 for SCDser; 2.1±0.3; 0.9±0.1%; P=0.01 for SCDHser without or with Necrostatin, respectively; n=7), although non-necroptotic death of CONneu incubated with SCDser was significantly higher than for CONneu incubated with CONser (1.1±0.2; 0.6±0.06%; P<0.0001; n=7; P<0.001). Conclusions. Characterization of circulating neutrophils demonstrated a higher incidence of immature cells in SCD individuals, indicating a shift in cell turnover and/or earlier release of leukocytes from the bone marrow. While SCDneu appear to demonstrate an increase in cell survival, the serum of SCD individuals seems to contain pro-apoptotic factors, as indicated by the stimulation of phosphatidyserine presentation and caspase-9 induction in leukocytes incubated with SCD serum. Necroptosis may contribute slightly to neutrophil cell death, independently of serum type, although apoptotic cell death seems to be increased by SCD serum. Data indicate that alterations in cell turnover or emigration from bone marrow may contribute to elevate leukocyte number and delay cell death in SCD; however cell death may be subject to modulation by a complex balance of both anti- and pro-apoptotic factors contained in the serum of SCD individuals.

0415 EYA3 SILENCING PROMOTES MODIFICATIONS IN THE EXPRESSION PATTERN OF GLOBINS GENES, HBF AND APOPTOSIS LEVELS SUGGESTING ITS PARTICIPATION IN ERYTHROID DIFFERENTIATION
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Background. Erythroid differentiation is a dynamic process in which a pluripotent stem cell undergoes a series of developmental changes that commit it to a specific lineage. These alterations involve changes in gene expression profiles. Previous results using SAGE identified 93 differentially-expressed genes during erythroid development. One of these genes, EYA3, a homologue of Eyes Absent in Drosophila, is a transcription cofactor with intrinsic phosphatase activity and its expression was observed to be high at the end of CD34+ differentiation and in human bone marrow. Aim. Evaluate effects of EYA3 gene silencing in the K562 (erythroleukemia cell) line after hemin induction, such as modifications of globins gene expression, apoptosis and fetal Hemoglobin (HbF) expression. Furthermore, we evaluated EYA3 gene expression using CD34+ cells and reticulocytes from sickle cell disease (SCD) patients, compared to a control group. Methods. Two different cultures from human K562 cells (1x10^6 cells/mL in DMEM, 10% FBS, penicillin/streptomycin, 5% CO2, 37°C) were transfected with control or EYA3 knockdown lentivirus (MOI=1.0). After proliferation and selection of positive cells with puromycin (2.0 µg/mL), cells were treated with 30µM hemin and collected after 0, 24, 48, 72 and 96h for gene expression and flow cytometry analysis. CD34+ hematopoietic cells from 4 control individuals were proliferated and differentiated into late stage erythroblasts. EYA3, α, β and γ-globin gene expression was analyzed by qRT-PCR and quantified using the Gnorm program. HbF expression and apoptosis were evaluated by flow cytometry. Results. Analysis of α, β and γ-globin gene expression shows that these genes are downregulated in K562 culture cells knockdowned for EYA3 compared with a control culture at 0, 24, 48, 72 and 96h after hemin addition (see Table 1). α-globin gene expression was observed to be high at the end of CD34+ differentiation and in human bone marrow. Table 1: Evaluation of EYA3 gene expression using CD34+ primary culture showed that the expression of this gene increases during erythroid differentiation (day 7: 0.98±0.03; day 10: 1.37±0.5; day 13: 1.2; P<0.05). Whilst SCDneu cultured in the presence of CONser pre-
iron overload in beta thalassaemia major (TM) enhances oxidative stress within the liver which is associated with the development of fibrosis that may progress to cirrhosis. The interactions between various resident hepatic cell populations and immune cells that lead to the establishment of fibrosis are complex and little information is available with respect to the possible alterations in serum levels and tissue expressions of fibrosis markers and contribution to the liver fibrosis in TM. Methods. This study was run in Turkey following the completion of the ICL670A0107E extension study in patients who completed core phase and continued with deferasirox during 4 year extension were included after consenting for participation in this locally run study. Liver biopsy specimens and simultaneously collected frozen serum samples of those patients were used. Serum concentrations of tenascin, collagen IV, tissue inhibitors of metalloproteinase (TIMP-1), and matrix metalloproteinase (MMP-1) levels were measured with enzyme-linked immunosassay kits. Liver iron concentrations (LIC) were measured by AAS. Fibrosis stage and inflammation grade were assessed in a blinded fashion by a single pathologist according to the Ishak (score 0-6, grade 0-18) system and iron stained and staged according to the Lichler (0-4x3). Paraffin sections from formalin fixed material were immunostained with antibodies against alfa-SMA, Collagen-4, TIMP-1 but not other pro-MMPs. TIMP-1 also binds to the catalytic domain of all soluble activated MMPs and inhibits their enzymatic activity. The aim of the study was to evaluate the serum levels of TIMP-1 and MMP-9 in patients with TM-related osteoporosis and explore possible correlations with bone remodeling and bone mineral density (BMD). Methods. Twenty-two patients with thalassaemia-induced osteoporosis (10M/12F; median age 42 years) were studied. Patients were blindly randomized to receive zoledronic acid at a dose of 4 mg, iv, in 15 min infusion, every 6 months (n=16) or to receive placebo every 6 months (n=6) for a period of one year. All patients were under oral calcium (Ca) and vitamin D administration during the treatment period. TIMP-1 and MMP-9 were measured at baseline and after 12 months of therapy using ELISA methodology (Oncogene Science/ Siemens HealthCare Diagnostics, Cambridge, MA, USA and R&D Systems, Minneapolis, MN, USA, respectively) along with determination of serum bone remodeling indices: i) bone resorption markers (C-telopeptide of type-I collagen (CTX), tartrate-resistant acid phosphatase isofrom-5b (TRAP-5b)), ii) bone formation markers (bone-alkaline phosphatase (bALP), osteocalcin, and C-terminal propeptide of collagen type-I (CICP)), and iii) osteoclast regulators [receptor activator of nuclear factor-kappa B ligand (RANKL), osteoprotegerin (OPG), and osteopontin]. BMD of the lumbar spine (L1-4), femoral neck (FN) and wrist (W) was determined using DXA, before and 12 months after treatment. The above bone markers were also evaluated in 30, age- and gender-matched, healthy controls. Results. At baseline, six patients (SM/5F; 27%) had elevated values of TIMP-1 (upper normal limit 459 ng/ml for males and 374 ng/ml for women). Furthermore, TM patients had increased values of MMP-9 (median and range: 628 ng/ml, 289-911 ng/ml versus 312 ng/ml, 113-514 ng/ml; p<0.001), CTX (p<0.001), bALP (p<0.001), CICP (p<0.005), sRANKL (p<0.02), and OPG (p=0.001) compared with controls. TIMP-1 serum levels correlated with OPG (r=0.461, p=0.031), sRANKL/OPG ratio (r= -0.485, p=0.028), bALP (p=0.049), p=0.026) and OPN levels (r=0.533, p=0.015). Patients with elevated values of TIMP-1 had increased L1-L4 z-score (median: -1.65, range: -2.5 to -1.5) compared to patients with normal values (median: -2.05, range: -4.4 to -1.2; p=0.042). MMP-9 correlated with CTX (r=0.476, p=0.03) and TIMP-1 (r=0.445, p=0.041). The increase of z-score of BMD did not correlate with other serum levels of TIMP-1 or MMP-9, although these patients experienced an increase of BMD in all measured sites. Summary/Conclusions. TIMP-1 serum levels are elevated in approximately 25% of patients with TM-related osteoporosis and associated with BMD. This increase may reflect a balance effect on the increased MMP-9 activity present in this condition. Larger studies will reveal if high TIMP-1 protects TM-induced bone loss and reveal MMP-9 as possible target for development of novel drugs against TM-induced osteoporosis.

ASSOCIATION STUDY BETWEEN SNPs IN GENES RELATED TO ADENOSINE SIGNALING AND DISTINCT CLINICAL MANIFESTATIONS IN SICKLE CELL DISEASE

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Background. Recent studies have demonstrated the role of high adenosine levels in priapism episodes in a mouse model of sickle cell disease (SCD). Interestingly, in addition to priapism, altered adenosine signaling (through four distinct receptors; A1, A2a, A2b and A3) has been implicated in several physiopathological processes that are asso-
cated to distinct clinical features observed in patients with SCD. Moreover, adenosine levels are partially controlled by its conversion into inosine, carried by the enzyme Adenosine Deaminase (ADA). Although SCD is characterized by a single base substitution resulting in a mutated β-globin (HbS), a broad spectrum of clinical manifestations and severity are observed in the patients. This variability reflects the distinct genetic background of these patients. Aims. To evaluate the potential association of some single nucleotide polymorphisms (SNPs), present in adenosine receptors and ADA genes, with clinical manifestations observed in SCD patients. Methods. DNA was extracted from peripheral blood samples collected from a total of 230 patients with Sickle Cell Disease (SS and SB), being assisted in the Regional Blood Center (RHCM). Samples were obtained after informed consent, following a protocol approved by the local ethics committee. Three SNPs were evaluated. The SNPs in the ADORA1 (C/T) alleles, rs165103) and ADORA3 (G/T alleles, rs35511654) genes were evaluated by Real Time PCR using TaqMan probes and primer, the SNP 968 G>T and 1007 C>T in the ADORA2B gene were identified and evaluated by sequencing, the SNP ADA*2 (Asp8Asn; G22A, GeneBank M13792) in the ADA gene was evaluated by restriction fragment length polymorphism (RFLP). The main clinical manifestations evaluated, and the age cutoff for inclusion in the study, were: acute chest syndrome (ACS), pulmonary hypertension (FH, >10 years old), priapism (>15 years old, male patients), bone disorders, such as osteoporosis (OD); >13 years old), and stroke (>5 years old). The software GENEPOP 3.4 was used to test for Hardy-Weinberg equilibrium and a Fisher exact test was carried to identify potential associations between polymorphisms and the clinical manifestations considered. Haplotype studies and corresponding statistical analysis (Tukey test) were carried using the softwares Arlequim (v3.1) and SAS (v9.13), respectively. Results. Significant differences were found for the 1007 C>T SNP of the ADORA2B gene, with patients in the group with acute chest syndrome showing an increased frequency of the T allele (p=0.052). For this same SNP a significant higher frequencies where of genotype C/C and C/T were found among patients with HD (p=0.015). For the SNP 968 G>T, we found a significant higher frequency of patients with the G/G genotype among patients with HD (p=0.0043). Haplotype studies, revealed a significant association with the manifestation of distinct clinical features, including, priapism, stroke and ACS. Summary. Our results indicate that distinct genes related to adenosine signaling may play a role as modifiers of severity in sickle cell disease.

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0419 ASSESSMENT OF OXIDATIVE STRESS IN PATIENTS WITH SICKLE CELL DISEASE

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Aims. Continuous Reactive Oxygen Species (ROS) production in individuals with Sickle Cell Disease (SCD) may alter their overall redox status and cause tissue damage. The aim of this study was to evaluate oxidative stress in patients with SCD. Patients and Methods. A total of 40 patients with SCD and 25 apparently healthy volunteers (control group) were enrolled in the study. Components of glutathione system (GSHtotal, GSSG and GSHreduced), vitamins A, C and E, SOD, antioxidant enzymes GPx and SOD, were significantly higher in patients with SCD compared to controls (p<0.001), while GSSG levels were significantly lower in patients with SCD compared to controls (p<0.001). Furthermore, patients with SCD have significantly higher total NO levels compared to controls, (p<0.001). FORT levels were significantly higher patients with SCD compared to controls, (p<0.001), while FORD levels were significantly lower in the patients with SCD compared to controls, (p=0.02). Red cells antioxidant enzymes GPx and SOD, were significantly higher in patients with SCD compared to controls, (p<0.001 and p<0.001, respectively). The above reported observations were consistent with the significantly lower plasma concentrations of the antioxidant Vitamins A, C, E and F in patients with SCD compared to controls, (p<0.01 and p<0.001, respectively). Conclusion. Since oxidative stress seems to play a major role in SCD, the development of novel therapies focused on free radical biology appears imperative. Hemolysis and oxidative stress might interact positively, and antioxidants supplementation may constitute valuable means for improved manifestations and outcomes.

0420 TUMOR NECROSIS FACTOR POLYMORPHISMS IN PATIENTS WITH AUTOIMMUNE HEMOLYTIC ANEMIA

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Aims. Tumor necrosis factor (TNF) is a multifactorial cytokine that is secreted by monocytes (TNF-α) or lymphocytes (TNF-β). TNF cytokines have numerous immunoregulatory effects as well as proinflammatory effects, and they are implicated in many inflammatory and autoimmune diseases, like inflammatory bowel disease, rheumatoid arthritis, systemic lupus erythematosus, immune thrombocytopenic purpura, etc. Autoimmune hemolytic anemia (AIHA) is the second most common autoimmune blood disorders. AIHA may occur as primary (idiopathic) or secondary to other lymphoproliferative or immune disease. The etiology of AIHA remains unclear, but both genetic and environmental factors are thought to play role in the development of the disease. Aims. The aim of our study was to investigate a possible association of some single nucleotide polymorphisms (SNP) in genes for tumor necrosis factor beta (TNF-β -252 G/A) and tumor necrosis factor alpha (TNF-α -308 G/A) with autoimmune hemolytic anemia. Methods. We have analyzed 60 adult patients with AIHA; 30 patients with idiopathic AIHA and 30 patients with secondary AIHA and chronic lymphocytic leukemia (CLL). Controls were 120 healthy individuals and 100 CLL patients without AIHA. DNA was isolated from peripheral blood mononuclear cells with standard phenol-chloroform extraction. Genotyping was performed by using PCR and RFLP methods. The distribution of genotypes and allele frequencies were compared between patients and controls using a chi-squared test or Fisher’s exact test. Results. Our results demonstrated that the G allele of the TNF-β (-252 G/A) was significantly more frequent among the patients with AIHA (n=60; G/G=14, A/G=23, A/A=23) compared to controls (n=120; G/G=16, A/G=55, A/A=49), p=0.048. This difference was even more significant when only CLL patients were compared to controls (n=120; G/G=16, A/G=55, A/A=49, p=0.048). We found that the A allele of the TNF-α (-308 G/A) was also more common in patients with AIHA (n=60; G/G=33, A/G=22, A/A=5) than in controls (n=120; G/G=95, A/G=23, A/A=2), p=0.002. This difference was more striking when only 60 patients were compared between them (n=120; G/G=16, A/G=55, A/A=49) versus CLL without AIHA (n=100; G/G=10, A/G=51, A/A=39), p=0.018. We found that the A allele of the TNF-α (-308 G/A) was also more common in patients with AIHA (n=60; G/G=33, A/G=22, A/A=5) than in controls (n=120; G/G=95, A/G=23, A/A=2), p=0.002. This difference was more striking when only 60 patients were compared between them (n=120; G/G=16, A/G=55, A/A=49) versus CLL without AIHA (n=100; G/G=82, A/G=17, A/A=1), p=0.0006. There was no significant difference in genotype distributions between CLL patients without AIHA and healthy control individuals for both genes.
(p=0.74 for TNF-β and p=0.83 for TNF-α). Conclusion. The obtained data indicate that the G allele of TNF-β (+252) and A allele of TNF-α (-308 G/A), which are both associated with increased TNF production and secretion, are more frequent in patients with AIHA than in controls, especially in the group of CLL patients with AIHA. These results implicate that these two polymorphisms may predispose to the development of autoimmune hemolytic anemia, especially in the group of patients with CLL.

0421
ERYTHROPOIESIS DISTURBANCE IN HEREDITARY SPHEROCYTOSIS CLINICAL OUTCOME
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Hereditary Spherocytosis (HS) is the most common non-immune hemolytic anemia in individuals of northern European ancestry (1/2000). In a previous study, we showed that high levels of erythropoietin (EPO) were not able to induce a proportional reticulocyte production in moderate HS patients that was observed in mild HS (Rocha S, Br J Haematol, 2005, 131, 534-542). In the present study, our aim was to evaluate the relationship between that erythropoietic disturbance and inflammation, in the clinical outcome of HS (mild, moderate and severe HS). We studied 82 unsplenectomized HS Portuguese patients, presenting mild (n=49), moderate (n=27) and severe (n=6) HS. We evaluated plasma levels of EPO, soluble transferrin receptor (sTfR), iron, transferrin, ferritin, folic acid, vitamin B12, C-reactive protein (CRP), granulocyte-monocyte colony stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, interferon (IFN)-γ, elastase and lactoferrin; determined reticulocyte count and the total and differential leukocyte counts, and calculated the reticulocyte production index (RPI). Mild HS patients showed a rise in EPO, sTfR, reticulocytes and RPI, reflecting a compensated hemolysis, and positive statistical significant correlations between EPO and sTfR, reticulocytes and RPI, were observed. In moderate and severe HS, in spite of significantly higher EPO, sTfR, reticulocytes and RPI than mild HS, these correlations were not observed. For all patients iron stores, folic acid and vitamin B12 were within normal values or were slightly higher. HS patients presented a low grade inflammation that was particularly enhanced in severe HS, as shown by the highest median levels of GM-CSF, CRP, TNF-α, IFN-γ and elastase and the lowest levels of iron and lactoferrin. Our data show HS as a disease linked to enhanced erythropoiesis that in the more severe forms (moderate and severe) is disturbed. Inflammation may contribute, at least in part, to that disturbance, especially in the severe cases of HS. This study was supported by a PhD grant (SFRH/BD/22442/2005) attributed to S.Rocha by FCT and FSE.

0422
MLPA ANALYSIS OF BETA GLOBIN GENE CLUSTER: DETECTION OF 41 ALTERATIONS (INCLUDING FIVE NOVEL DELETIONS) IN SPANISH POPULATION
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Background. Genetic alterations in the beta globin cluster can lead to a variety of diseases such as High Persistence of Fetal Hemoglobin (HPFH), deltatheta thalassemia (β thal), beta thalassemia (β thal), and more. Sequencing the globin genes is the more suitable method for the identification of point mutations or small deletions. However, large deletions are common cause of disease, due to the elimination of entire genes or regulatory elements dispersed in the beta cluster. There are PCR methods designed to detect nothing but a few of this known deletions. Thus, those screening techniques can not be used in the search of novel alterations and the molecular diagnosis becomes difficult in a considerable proportion of the cases. Recently, the development of the Multiplex Ligation-dependent Probe Amplification (MLPA) technique has solved this problem, and now it is possible a quick identification of any copy number variation in the beta globin cluster. Aim. This is a descriptive work showing the results of the analysis by MLPA method of cases suspected to carry an alteration in the beta globin cluster. Methods. This study included 66 patients with a phenotype of HPFH, βthal or β thal. The existence of point mutations or small deletions/duplications in the β gene was ruled out by sequencing. The MLPA technique is based on the quantitative amplification and a subsequently fragment analysis of multiple probes hybridized across a region of interest. This method allows for a genetic profile showing the copy number variation of those targets. A deletion is detected when a reduction of the amount of amplified product of several consecutive probes is observed in the gel. Here we used a commercial kit (MLPA kit P102-B1 HBB, MRC-Holland) that contains 28 probes designed to detect copy number changes in the beta cluster, from LCR to 10Kb down-stream of β globin gene, spanning more than 80Kb. Results. Sixty-three percent of the patients are carriers for an alteration that can satisfactorily explain their clinical manifestations. A total of 41 alterations contain a deletion or duplication in the β cluster. Ten different genetic alterations have been detected in this study; five of them has not been previously described in scientific publications and are novel. Twenty-five patients have shown a normal genetic profile. The alteration most frequently found is the (□β) Spanish deletion. A total of 31 patients are heterozygote carriers for this deletion. Duplication in the region of gamma genes has been found in two cases. All the other alterations detected here have been found in only one case (detailed view in figure 1). Finally, we are currently designing long range PCRs for the identification of the breakpoints of the novel deletions mentioned above. Conclusions. Almost all the deletions detected have been found in only one case. The results support the idea of the existence of a high molecular heterogeneity for the alterations in the β cluster. Within this scenario, MLPA analysis means an improvement of the molecular diagnosis and the genetic counseling applied to this group of diseases.

Figure 1.
OUTCOME OF SECOND ALLOGENEIC HCT FOLLOWING RELAPSE OF HAEMATOLOGICAL MALIGNANCIES AFTER FIRST ALLOGENEIC HCT: EXPERIENCE FROM THE GETH (GRUPO ESPANOL DE TRASPLANTE HEMATOPOYETICO)

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Background. Disease relapse is the most frequent cause of treatment failure following allogeneic haematopoietic cell transplantation (allo-HCT), and carries a very poor prognosis. A second allo-HCT may be the only curative option for the majority of these patients. Patient and transplant factors that may associate with the outcome of a second allo-HCT are required for clinical-decision making in such high-risk patients. Aims. We performed a retrospective analysis of adult patients receiving a second allo-HCT for disease relapse after a prior allo-HCT reported by GETH centres. Our aim is to analyze our experience in this setting, and to identify factors associated with patient outcome. Results. We present data on 66 patients who underwent a second allo-HCT for disease relapse in Spain between 1990 and 2010 (median year 2003), with a median follow-up for survivors of 68 months (8-224): median age 38 years (range 14-69); 35 male and 31 female; initial diagnosis AML 25 patients, ALL 13 patients, MDS/MPD 11 patients, CML 11 patients, lymphoproliferative disorder 6 patients; 23 (35%) cases of myeloablative conditioning. Donors were related in 55 cases. Also, 11 second allo-HCT were performed with new donors different from the donors in the previous allo-HCT. Only 4 cases had T-cell depletion. Median time from the first to the second allo-HCT was 21 months (1-170). The cumulative incidence of non-relapse mortality was 26.2%. The median overall survival (OS) was 315 days (95% CI, 176 - 454), with an OS at 1, 3 and 5 years of 38%, 31% and 31%, respectively. OS survival was significantly better in patients who underwent second allo-HCT with low disease burden (complete response, good partial response, or chronic phase; 61% at 1 year, 51% at 3 years) than in patients in active relapse or progression (22% at 1 year, 17% at 3 years; p<0.001). Patients who relapsed early after the first allo-HCT and required a second allo-HCT <1 year after first HCT also had a poorer outcome (OS 17% at 1 year, 9% at 3 years) than those having the second allo-HCT 1 year or longer after the first one (50% at 1 year, 43 at 3 years; p=0.001). Time to second allo-HCT and disease status also associated with the probability of progression free survival in this series (p=0.001 and p=0.002, respectively). Type of donor (related versus unrelated and new donor versus same donor) and type of conditioning (myeloablative versus non-myeloablative) had no statistically significant association with patient outcome. Conclusions. The experience from GETH reported here shows that over 30% of patients who relapse after an allo-HCT can achieve long-term survival of 5 or more years following a second allo-HCT. Also, our data suggest that disease status at HCT and time to relapse between first and second HCT are two significant prognostic factors for survival outcome. The indication of a second allo-HCT should be thoroughly discussed for individual cases with early relapse and active disease. Donor type does not appear to influence the outcome.
ated cells. Therefore, we hypothesized that the impact of graft composition on acute GVHD in children undergoing BM transplantation could be different and a threshold dose of cells that affects GVHD can be defined. This is the first study prospectively evaluating the impact of the CD3+ and CD34+ cell doses infused on GVHD in children undergoing HLA-identical sibling BMT for nonmalignant diseases to date. Aims. Assessing the impact of graft composition on aGVHD in children undergoing HLA-identical BMT from sibling donors. Methods. Between 2004 and 2010, 92 patients with median age of 8 years (range, 1.6-17) were given a bone marrow graft for thalassemia. The preparatory regimens for class 1 and class 2 patients (n=49) consisted of BUCY200 ± thiotepa, and for class 3 patients (n=45) of BUCY160 ± thiotepa (preceded by cyclophosphamide). The immunosuppression regimen with BUDC, azathioprine and fludarabine. From June 2006 onwards, all patients were given targeted i.v.Busilvex (Pierre Fabre Medicament, France). As GVHD prophylaxis patients received CSA, a short course of MTX and methylprednisolone. Results. The median of 4.6x10^6/kg (range 1.3-10.8) total nucleated cells (TNC), 7.2x10^6/kg (range 0.8-35) CD34+ cells, and 55.5x10^6/kg (range 3.8-208) of CD3+ cells were infused. There was a weak correlation (Spearman’s test) between CD34+ and CD3+ cell doses (p=0.05 and 0.06, respectively). Cumulative incidence of grade 2-4 and 3-4 aGVHD was 38% (95% CI: 25-44) and 9% (95% CI 4-16), respectively. In univariate analysis only CD3+ and CD34+ cell doses above or equal to the median value were significantly associated with grade 2-4 aGVHD (49% vs. 20%; p=0.005 and 46% vs. 25%; p=0.01, respectively). Multivariate analysis confirmed that high CD3+ (HR, 4.6; p=0.010) and CD34+ (HR, 4.5; p=0.011) cell doses were major risk factors for grade 2-4 aGVHD. We further examined the effect of CD3+ and CD34+ cell doses on grade 2-4 aGVHD and found a minimum threshold for CD3+ (4.3x10^6/kg) and CD34+ (0.8x4x10^6/kg) cells above which the incidence of grade 2-4 aGVHD is significantly increased (8% to 38%-54% and 5% to 41%, respectively). Cumulative incidence of extensive GVHD was 10% (95% CI 5-18). Conclusion. This study for the first time demonstrated that high doses of CD3+ and CD34+ cells within the graft are risk factors for grade 2-4 aGVHD in children with thalassemia undergoing HLA-identical BMT, and defined a minimum threshold dose for these cells above which the incidence of aGVHD significantly increases. These data indicate that patients receiving CD3+ and CD34+ cell doses beyond minimum threshold should be given additional immunosuppressive agents.

**0426**

THE RISK FACTORS FOR EBV VIREMIA EARLY AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Objective. To identify the risk factors for Epstein-Barr Virus (EBV) viremia early after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Patients and Methods. Between January 2007 and January 2009, 89 patients after allo-HSCT (75, matched sibling 66) with continuous monitoring plasma EBV-DNA were studied. Conditioning regimens were BUCY/Flu or CY/Flu TBI mainly. ATG was added in both haploidentical and unrelated donor transplants. Cyclosporine, mtermoxat and mycophenolate mofetil were employed for GVHD prophylaxis. Serum EBV status of donor and recipient pre-HSCT was determined by ELISA. The levels of plasma EBV-DNA were monitored with real-time quantitative polymerase chain reaction (RQ-PCR) 1 to 2 times weekly in the first 3 months after allo-HSCT. EBV viremia was diagnosed when plasma EBV-DNA was more than 5 x 10^2 copies/ml but without symptoms. Acyclovir (10mg/kg or 500mg/m^2 IV q8h) was administered for pre-emptive therapy and immuno-suppressants were decreased if possible. Results. Total 33 patients (11.9%) developed EBV viremia with the median time at day 44 (day 19 to day 84). The incidence of EBV viremia were 15.5%, 20.0%, 0% in haploidentical, unrelated, matched sibling transplant, respectively. There was no significant difference in incidence of EBV viremia between haploidentical and unrelated transplants (p=0.09), but much less EBV viremia was seen in matched sibling transplant (p=0.001). Twenty of 33 patients with EBV viremia (60.6%) had complete response to the pre-emptive therapy with acyclovir. The median time to reach plasma EBV-DNA negative was 21 days (14 to 56 days). The median duration of pre-emptive therapy was 21 days (14 to 60 days). Both univariate and multivariate analysis indicated that haploidentical and unrelated transplants, acute GVHD were the risk factors for EBV viremia. Two-year overall survival in the patients with EBV viremia was significantly lower than that without EBV viremia (54.2% vs. 72.1%, p=0.006). Conclusions. Our large clinical study has demonstrated that it is not necessary to monitor plasma EBV-DNA in matched sibling transplant. The incidences of EBV viremia are similar between haploidentical and unrelated transplants. Majority of patients with EBV viremia benefits from the pre-emptive therapy with acyclovir. Haploidentical and unrelated transplants, acute GVHD are the risk factors for EBV viremia early after allo-HSCT which has negative impact on survival.

**0427**

PROGNOSTIC RELEVANCE OF MINIMAL RESIDUAL DISEASE (MRD) PRIOR TO AUTOLOGOUS TRANSPLANTATION ON LONG-TERM FOLLOW-UP IN ACUTE MYELOID LEUKEMIA (AML) ON BEHALF OF ROME TRANSPLANT NETWORK (RTN)

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Background. autologous stem cell transplantation (ASCT) is currently considered a therapeutic option improving outcome of patients with acute myeloid leukemia (AML). Up to now, it is controversial in which risk patient subgroup it may play a role as post-consolidation therapy. Therefore, risk assessment should be integrated with other parameters to point out this issue. Assessing the impact of MRD prior to ASCT has a high prognostic value in terms of disease free survival (DFS), relapse free survival (RFS) and overall survival (OS). Both univariate and multivariate analysis indicated that MRD, as a continuous variable, is statistically significant (see Figure 1). The risk assessment at diagnosis has not been considered in these curves. In order to evaluate the prognostic significance of MRD prior to ASCT, its value detected by flow-cytometry (FCM) has been retrospectively analyzed in AML patients followed on a long-term follow-up. Patients and Methods. 89 AML pts with median age of 50 years (range 20-75) undergoing ASCT (June 1995-October 2009) in four Institutions participating to the Rome Transplant Network (RTN), a metropolitan transplant network, have been analyzed. The cytogenetic risk groups were distributed as follows: 46 (63%) intermediate, 12 favourable, 15 adverse; 7 (12%) pts were Flt3-ITD positive. Patients received ASCT in first CR after standard induction- consolidation chemotherapy according to AML EORTC-GIMEMA trials. Bone marrow MRD by FMC was determined prior to ASCT using a quantitative real-time polymerase chain reaction (RQ-PCR) 1 to 2 times weekly in the first 3 months after allo-HSCT. EBV viremia was diagnosed when plasma EBV-DNA was more than 5 x 10^2 copies/ml but without symptoms. Acyclovir (10mg/kg or 500mg/m^2 IV q8h) was administered for pre-emptive therapy and immuno-suppressants were decreased if possible. Results. Total 33 patients (11.9%) developed EBV viremia with the median time at day 44 (day 19 to day 84). The incidence of EBV viremia were 15.5%, 20.0%, 0% in haploidentical, unrelated, matched sibling transplant, respectively. There was no significant difference in incidence of EBV viremia between haploidentical and unrelated transplants (p=0.09), but much less EBV viremia was seen in matched sibling transplant (p=0.001). Twenty of 33 patients with EBV viremia (60.6%) had complete response to the pre-emptive therapy with acyclovir. The median time to reach plasma EBV-DNA negative was 21 days (14 to 56 days). The median duration of pre-emptive therapy was 21 days (14 to 60 days). Both univariate and multivariate analysis indicated that haploidentical and unrelated transplants, acute GVHD were the risk factors for EBV viremia. Two-year overall survival in the patients with EBV viremia was significantly lower than that without EBV viremia (54.2% vs. 72.1%, p=0.006). Conclusions. Our large clinical study has demonstrated that it is not necessary to monitor plasma EBV-DNA in matched sibling transplant. The incidences of EBV viremia are similar between haploidentical and unrelated transplants. Majority of patients with EBV viremia benefits from the pre-emptive therapy with acyclovir. Haploidentical and unrelated transplants, acute GVHD are the risk factors for EBV viremia early after allo-HSCT which has negative impact on survival.

Figure 1.
Figure 1.

**A PROSPECTIVE RANDOMIZED TRIAL COMPARING PHLEBOTOMY AND DEFERASIROX FOR THE TREATMENT OF IRON OVERLOAD IN PAEDIATRIC THALASSEMIA MAJOR PATIENTS CURED BY STEM CELL TRANSPLANTATION: 6-MONTH FOLLOW-UP**

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**Background.** Patients with β-thalassaemia major who have undergone curative haematopoietic stem cell transplantation (SCT) are at increased risk of iron overload because of previous transfusion therapy. There are limited data on post-SCT iron removal achieved with phlebotomy or iron chelators. *Aims.* To compare efficacy, safety and convenience of phlebotomy versus deferasirox for the treatment of iron overload in children with thalassaemia major after allogeneic SCT. **Methods.** Study LB03T is an ongoing prospective, 1-year, randomized trial in paediatric patients with thalassaemia major who have undergone allogeneic SCT. Chelation-naïve, hepatitis B- and C-negative patients aged 2–18 years with iron overload (serum ferritin >500ng/mL on ≥2 monthly occasions, and liver iron concentration [LIC] >5mg Fe/g dry weight [dw] determined by MRI) were eligible. Patients were randomized to phlebotomy (6mL/kg blood/2 weeks) or deferasirox (10mg/kg/day starting dose; 5mg/kg/day adjustments up to a dose of 20mg/kg/day according to 3-monthly ferritin trends were allowed). Compliance with phlebotomy was determined by the ratio of phlebotomy performed over planned phlebotomy; compliance with deferasirox was determined by missed tablet counting. **Results.** 28 patients were enrolled and randomized to phlebotomy or deferasirox. Two patients randomized to phlebotomy refused treatment. Baseline parameters for 26 patients were comparable between treatment groups (Table). Mean patient age: 12.6 years, mean follow-up: 6.8 months. For patients with serum ferritin <1000ng/mL at baseline, the efficacy of deferasirox at 10mg/kg/day and phlebotomy was similar (Table); for patients with serum ferritin ≥1000ng/mL, the absolute median change was non-significantly greater with phlebotomy. For TIBC, the increase with deferasirox was significantly greater than with phlebotomy in all groups. The absolute increase in haemoglobin was -0.12 and -0.59g/dL after deferasirox and phlebotomy, respectively. Parents of 13/14 children randomized to phlebotomy noted a desire to switch to deferasirox.

**Summary/Conclusions.** For post-haematopoietic SCT patients with serum ferritin <1000ng/mL, phlebotomy and deferasirox (10mg/kg/day) were equally effective in ferritin reduction; in patients with serum ferritin ≥1000ng/mL, phlebotomy reduced serum ferritin to a greater extent (difference did not reach significance between treatment groups). This highlights that a starting dose of 10mg/kg/day may not be sufficient to reduce iron loading in such patients, and that earlier and appropriate dose adjustments should be carried out. Deferasirox increased TIBC to a more statistically significant extent than phlebotomy. Deferasirox was well tolerated; the majority of parents with children receiving phlebotomy noted a desire to switch to deferasirox.

**0429**

**EPIDERMAL LANGERHANS CELLS IN THE CONTEXT OF FULL AND REDUCED INTENSITY CONDITIONING**

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The interaction of residing host-derived dendritic cells (DC) and donor T cells is a crucial step in the induction of acute graft versus host disease (GVHD). In particular, epidermal Langerhans cells (LC) of the recipient persist after allogeneic haematopoietic stem cell transplantation (HSCT). Allo-reactive T cells mediate GVHD but also promote the switch from host to donor LC. We investigated on the LC chimerism early after transplantation in patients who had been treated with a reduced intensity conditioning (Fludarabine / Melphalan) combined with in vivo T-cell depletion mediated by high-dose alemtuzumab (100mg) prior to HSCT. In addition, we compared the impact of this conditioning regimen on LC-density with that of a 12Gy total body irradiation (TBI)-based full intensity conditioning regimen. Epidermal skin layers were prepared from 6 mm punch biopsies and split for further immunofluorescent staining as well as for the generation of single cell suspensions. CD1a/MHC-class II-positive LC were analyzed and subsequently sorted by flow cytometry. LC frequency among the isolated epidermal cells was analyzed by flow cytometry prior to conditioning, on the day of transplantation as well as on day +20. In addition, LC density was semiquantitatively assessed by confocal laser-microscopy on stained epidermal sheets. Donor chimerism was measured by STR-based assays on LC isolated on day +20. Despite of a low LC-density on day +20 compared to that before transplantation, LC could be isolated in 51/52 patients. The numbers of isolated cells ranged from 2 to >1000. In samples with >300 isolated cells, these were re-analyzed by flow-cytometry and showed a purity of >90%. Chimerism analyses led to valid results in 39 samples. Of 19 patients who suffered from an
early GVHD after transplantation, 10 patients (53%) had predominantly donor-derived LC as early as day +20 after HSCT. In contrast, of 20 patients without primary GVHD, only 3 (15%) predominantly had donor-derived LC. The 20 patients without GVHD subsequently received prophylactic CD8-depleted donor-lymphocyte infusions (DLI). DLI induced acute GVHD in 11 patients, but we did not find a correlation between LC chimerism and the incidence of acute GVHD. The relative LC-density, calculated as the quotient of LC frequency among epidermal cells before and after conditioning was 60% (+/-30%) after Flu/Mel/Alenzutuzumab (n=5) and 6% (+/-3.5%) TBI-based conditioning (n=4). These results were confirmed by manual counting of LC in stained epidermal sheets. We have established a sensitive and feasible method enabling us to investigate LC chimerism and follow LC chimerism prospectively. Early after SCT following a Flu/Mel/Alenzutuzumab conditioning, the majority of patients still have predominantly host-derived LC. However, the predominant donor-LC chimerism in patients with primary GVHD on day +20 supports the hypothesis that persisting LC are relevant targets of allo-reactive T cells.

To investigate the impact of the conditioning regimen on LC density. Further studies will focus on the persistence of host-LC particularly after re-conditioning and the influence of CRT on LC density. We have established a sensitive and feasible method enabling us to investigate LC chimerism and follow LC density. We have established a sensitive and feasible method enabling us to investigate LC chimerism and follow LC density. We have established a sensitive and feasible method enabling us to investigate LC chimerism and follow LC density.
in the two groups respectively. The median follow-up after transplant was 45 (2-127) and 16 (3-39) months in the first and second group respectively. The cumulative incidence of acute graft versus-host disease (GVHD) was significantly higher before 2006 (47% vs 24%, p=0.0584). The cumulative incidence of chronic GVHD was also different (58% vs 30%; p=0.0241). The estimated probability of non-relapse mortality (NRM) at day 100 was 12% in the first group vs 0% in the second group transplanted after 2006. The one and two years NRM was 18% vs 25% (p=0.557). The overall survival (OS) at two years was 60% vs 70% in the first and second group respectively (p=0.1784). The progression-free survivals (PFS) was significantly different at 2 years, 45% before 2006 compared to 65% after 2006 (p=0.056). The PFS median not reached in the second group compared to 22 months before 2006 (p=0.1811). Conclusion. We documented a lower incidence of acute GVHD and NRM associated with a higher CR rate as well as significantly improved survival and relapse incidence since the introduction of novel reduced-intensity preparative regimens and peri- and post-transplantation strategies. These results suggest that enhancing the graft-versus-myeloma effect is of key importance for managing high-risk MM patients.

0433 EARLY PERIPHERAL BLOOD AND T CELLS CHIMERISM DYNAMICS AFTER SINGLE CORD BLOOD TRANSPLANTATION WITH CO-INFUSION OF CD34+ CELLS FROM A THIRD PARTY DONOR PREDICTS CB GRAFT FAILURE

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Background. Umbilical cord blood (CB) transplant from unrelated donors has been increasingly used as an alternative stem cell source for patients with hematological malignancies lacking HLA-matched adult donors. The co-infusion of mobilized and selected CD34+ cells from a non-HLA-identical donor (dual transplant) has shown to reduce the period of posttransplant neutropenia and related early morbidity and mortality of single CB transplantation. The aim of this study was to analyze the predictive value of early posttransplant peripheral blood (PB) and T lymphocytes (TL) chimerism analysis after dual transplant regarding CB engraftment or failure. Patients and Methods. 15 patients with high risk disease underwent 16 dual transplants between 2004 and 2011. Chimerism analysis was performed weekly after graft infusion until complete chimerism was achieved by STR-PCR (AmpFlSTR SGM Plus; Applied Biosystems) in PB and TL purified using immunomagnetic technology (CD34+, Milteny Biotec). Complete chimerism (CC) was defined as <1% recipient in PB and <5% in leukocyte lineages (95% purity of enriched samples). Results. From the 16 transplants, 12 (Figure 1a-l) showed engraftment (>500 TNC) in a median of 16 days (11-28) reaching full CB chimerism in a median of 24 days. Early posttransplant PB chimerism analysis showed increasing percentages of CB cells in 8 cases (Figure 1a-h). Only 2 cases showed low (<15%) percentages of CB cells in the first sample (day +14), although both showed an increase in the second determination (day +21). In the remaining 4 cases the proportion of CB cells remained stable or slightly decreased from day +14 to day +21 (Figure 1i-l), however TL chimerism showed a significant increase or remained near 100% CB cells in both determinations. On the other hand, 4 out of the 16 transplants experienced primary CB graft failure (Figure 1m-p). Three of them showed low percentages of CB cells in PB (<15%) on day +14 with a further decrease in the second sample. The fourth case showed an initial high proportion of PB CB cells with a significant decrease in the following sample (TL chimerism not available). Conclusions. Early posttransplant chimerism dynamics in PB and TL can predict CB engraftment or failure in dual transplants. Initially low percentages of CB cells in PB without an increase within the first month post-transplant as well as a decrease in the proportion of PB CB cells without an increase in TL seem to correlate with CB failure. Therefore, an early significant proportion of CB in TL associates with CB engraftment irrespective of the dynamics of chimerism in PB.

0434 TREATMENT OF STEROID RESISTANT GRADE II TO IV ACUTE GVHD BY INFUSION OF MESENCHYMAL STROMAL CELLS EXPANDED WITH HUMAN PLASMA AND PLATELET LYSATE - A PHASE I/II STUDY

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Introduction. For numerous malignant and non-malignant hematological diseases allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy. Despite multiple improvements in the last decade in the field of HSCT, acute graft versus host disease (aGVHD) remains a life-threatening complication and reduces substantially efficacy of HSCT. In particular, the outcome of patients with severe steroid-resistant aGVHD is very poor. Therefore, it remains important to search for new therapeutic strategies for treatment of GVHD. Objective. Feasibility of the generation and efficacy of mesenchymal stroma cells (MSCs) generated with fetal calf serum (FCS) has been suggested recently. However, FCS is a putative source of prions and virus transmission. Therefore, the feasibility of the generation of MSCs expanded with human plasma and platelet lysate (hPPL) was tested as well as the feasibility and safety of the application of hPPL-MSCs in patients with steroid-refractory aGVHD. Method. In an open-label, non-randomized prospective phase I/II study MSCs were extracted from the bone marrow of healthy volunteers, expanded with hPPL, and stored. Patients with steroid-refractory aGVHD grade II to IV were treated with ~2x10^6/kg hPPL-MSC. Response rate, transplantation-related deaths, and other adverse events were assessed for up to 12 months after the last infusion of the cells. Results. Between January 2009 and December 2010, 20 patients were included, 2 patients drop out, and 18 patient were available for further analysis: 5 children and 13 adults. Median age was 52.5 years (range 1.3-65.9). Organs involved in aGVHD were the skin (67%), the gastrointestinal tract (88%) and the liver (28%). Overall grade was II for 4 (22%), III for 13 (72%), and IV for 1(6%) patients. 1 patient received one infusion, all other patients received two or more infusions. No patient had side-effects during or immediately after infusions of the hPPL-MSC. Median follow-up was 5.5 months (range 0.53-12). Complete overall response was observed in 11 patients (61%) after a median of 65 days (range 10-184 days). The overall survival was significantly better in responders when compared to non-responders (p<0.001). Of the 11 patients who reached a CR, 8 patients relapsed approximately 2 months after reaching CR (median 59 days, range: 1-244). Three children relapsed with clinical signs of an allo-immune-lung, auto-immune-cytopenia or limited cGVHD and all 5 adults relapsed with GVHD of the gut (median 98 days after reaching CR, range: 55-302 days). However, GVHD of the gut was then again sensitive to steroids. Overall, 7 patients died, 4 due to progression of...
we transplanted 33 consecutive patients with MM.

Methods. Thirteen patients (39%) (Group 1) and 20 patients (61%) (Group 2) had unrelated and related donor respectively. The median age was 48 years (39-63) in the first group and 56 years (40-67) in the second group. Thirty two patients (97%) received one or more autologous transplantation. The disease status at transplantation was Complete Remission (CR) or VGPR in (15%) vs (40%); Partial remission (PR) in (77%) vs (55%); progression or refractory disease in (8%) vs (5%) in the first and second group respectively (p=0.1770). stem cell source was peripheral blood stem cells (PBSC) in all patients in the related donor group and in 10 patients (77%) in the second group, the other 2 patients (15%) received cord blood cells. Twenty five patients (Group 1: N=4 (31%); Group 2: N=4 (20%) were treated with a RIC regimen and infusion of hPPL-MSCs in steroid-resistant aGVHD, 1 patient due to abdominal bleeding and 2 due to sepsis. Conclusion. Generation and infusion of hPPL-MSCs in steroid-resistant aGVHD during tapering or cessation of immunosuppressive drugs become again sensitive to the treatment with steroids.

0435
ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPANTATION WITH RIC CONDITIONING IN PATIENTS WITH HIGH RISK MULTIPLE MYELOMA: COMPARATIVE ANALYSIS OF OUTCOMES BETWEEN UNRELATED AND RELATED DONOR
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The purpose of this study was to assess the results of allogeneic stem cell transplantation (Allo-SCT) after reduced-intensity conditioning (RIC) from an unrelated donor in patients with high-risk multiple myeloma (MM) in a single centre. From January 2007 to January 2010 we transplanted 33 consecutive patients with MM. Methods. Thirteen (39%) (Group 1) and 20 patients (61%) (Group 2) had unrelated and related donor respectively. The median age was 48 years (39-63) in the first group and 56 years (40-67) in the second group. Thirty two patients (97%) received one or more autologous transplantation. The disease status at transplantation was (Complete Remission (CR) or VGPR in (15%) vs (40%); Partial remission (PR) in (77%) vs (55%); progression or refractory disease in (8%) vs (5%) in the first and second group respectively (p=0.1770). stem cell source was peripheral blood stem cells (PBSC) in all patients in the related donor group and in 10 patients (77%) in the second group, the other 2 patients (15%) received cord blood cells. Twenty five patients (Group 1: N=4 (31%); Group 2: N=4 (20%) were treated with a RIC regimen and infusion of hPPL-MSCs in steroid-resistant aGVHD, 1 patient due to abdominal bleeding and 2 due to sepsis. Conclusion. Generation and infusion of hPPL-MSCs in steroid-resistant aGVHD during tapering or cessation of immunosuppressive drugs become again sensitive to the treatment with steroids.

0436
Efficacy and long-term outcome of intervention forure red cell aplasia (PRCA) following allogeneic hematopoietic stem cell transplantation from major ABO-incompatible donors
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Background. No standard of care for PRCA following allogeneic hematopoietic stem cell transplantation (HSCT) from major ABO-incompatible donors has been established. Aims. The primary objective of the present study was to learn the efficacy and long-term outcome of intervention for post-transplant PRCA. Methods. The present study was conducted as a retrospective observational study and approved by the IRB and the Japan Society for Hematopoietic Cell Transplantation. The patient cohort was selected from the registry database of the Transplant Registry Unified Management Program (TRUMP) that covered both adult and pediatric transplantation using all kinds of graft sources in Japan. One-hundred forty-five recipients who achieved engraftment with delayed recovery of erythropoiesis and survived >6 months after transplantation without disease relapse were selected from 2,846 records of major ABO-incompatible allogeneic transplantation during 2003 to 2007. The questionnaires were sent to the transplant centers and the response rate was 68.3% (99/145 recipients). Forty-eight recipients were identified as having PRCA, and then the detailed transplant data of 46 out of those 48 recipients could be collected. Results. The grafts were bone marrow and blood stem cells in 33 and 13 patients, respectively. No patient with PRCA was reported after cord blood transplantation. Donors were related in 24 patients, and unrelated in 22. Incompatible hemagglutinins were anti-A in 28 patients, anti-B in 12, and both in 6. Treatment of PRCA except for transfusion was performed in 22 patients (intervention group) but not in other 24 patients (non-intervention group). Response to the primary treatment was observed in 2 out of 8 patients who had been rapidly tapered calcineurin inhibitors (2CR), and 6 out of 12 patients receiving corticosteroid (5CR, 1PR). None of the patients receiving rituximab (n=1) or erythropoietin (n=1) responded. Secondary therapy including rituximab, additional immunosuppressants, or DLI was given in 8 patients with 50% response rate. Overall response rate of intervention was 54.5%. Four out of 10 patients who did not show any responses to intervention spontaneously became transfusion-independent. Days from the diagnosis of PRCA to recover reticulocytes >1% and cumulative doses of RBC transfusion during the period were not significantly different between the two groups. Incompatible hemagglutinin titters at diagnosis of PRCA were not different, either. Strikingly, the Kaplan-Meier estimate of the survival demonstrated the inferior survival of the intervention group (log-rank 0.040). Eleven and 2 deaths were observed in the intervention and the non-intervention groups, respectively. Infections accounted for the death of 7 patients in the intervention group. Univariate analysis identified the 5 variables influencing the OS, and...
Table 1. Table 2.

0437 REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION IN YOUNG PATIENTS WITH VERY HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA (VHR ALL)

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Background. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) with reduced intensity conditioning regimens (RIC) has become a well-established approach in adult patients with hematologic malignancies. The role of this type conditioning in pediatric cancer has yet to be defined. The aim: to compare efficacy of reduced intensity conditioning (RIC) and myeloablative conditioning (MAC) for allo-HSCT in children with acute lymphoblastic leukemia (ALL) from different centers in Europe.

Methods. The current study included 98 ALL patients (pts) from 1 till 21 y.o. (median 12 y.o.) who underwent allo-HSCT between 12/2000 and 12/2010. Indication for allo-HSCT in children and adolescents was VHR ALL: late relapse, aGVHD-5; infection -2; transplant related toxicity -2, non-engraftment -1. Five from 28 pts after MAC allo-HSCT in relapse either III or IV CR are in CR (1-104 months; mediana 44 months). Other pts died - relapse (13), infection (7), aGVHD (3), disease progression (2), graft versus host disease (3), GVHD (1), patient refusal (1). RIC allo-HSCT of VHR ALL in CR pts ≤ 21 yo is effective and comparable with MAC allo-HSCT. These results make new approaches for pts in VHR ALL in CR, indicate sensitivity to immunoadaptive therapy and produce the base for clinical trials.

0438 RESULTS OF A MULTICENTRIC EXPERIENCE OF PLERIXAFOR USE IN HEMATOPOIETIC STEM CELLS MOBILISATION


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4 Hôpital Saint-Vincent de Paul, Lille, France
5 Hématologie Thérapie Cellulaire Hôpital Bretonneau, CHRU de Tours, Tours, France
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Background. Plerixafor the major inhibitor of the CXCL12 receptor has been largely used in the context of hematopoietic stem and progenitor cells mobilization (HSCM) since its approval in 2009 in Europe. In France, its main indication remains for poor mobilizers patients with lymphoma and or myeloma. Aims and Methods. We retrospectively reviewed the data collection of 65 patients assigned to autologous stem cells transplantation (ASTC) and who underwent their apheresis at the French Blood Institute between 2008 and 2010. All patients who received Granulocyte-colony stimulating factor (G-CSF: 10 micrograms/kg/dose subcutaneously daily × 4 days) during mobilisation attempt + Plerixafor (0,24 mg/kg/dose subcutaneously daily, after at least the fourth dose of G-CSF) were evaluated. Additional doses of G-CSF and Plerixafor for subsequent apherase sessions were administered using a COBE Spectra separator. Results. Baseline characteristics of the patients were: median age = 57 yrs (range, 12-67); sex ratio M/F = 1:1,3; haematological malignancies = 58; non-haematological malignancies = 7; 3 patients received high dose cyclophosphamide during mobilisation setting median lines of chemotherapy = 2 (range, 1-6). Plerixafor was well tolerated and no severe side effects reported. Patients had a median of 2 mobilisation attempts (range, 0-4). Sixty three percent of patients achieved the aim of ≥ 2x106 CD34+ cells/kg collection after a median number of apherase sessions of 2 (range, 1-5). The median level of CD34+ cells in the peripheral blood (pCD34+) at day 1 of apheresis after Plerixafor injection was 19/ul (range, 0-145), 7,3 hours (range, 4,3-11,5) prior to apheresis. The median yield of CD34+ cells was 1,8x106/kg (0,1-1,65) after the first session of apheresis (Table 1). Interestingly, Plerixafor could lead 58 % of patients
to a pCD34+ level ≥ 20 /µl and collection of ≥ 3x106 CD34+cells/kg during only 1 session of apheresis. Moreover, the median level of pCD34+ available for 21 patients among the 65 before and after injection of Plerixafor was 4/µl (range, 0-16) and 12,5/µl (range, 0-97) respectively after a 3,2-fold median expansion (range, 0-16,2). At the day of analysis, twenty patients had already undergone ASCT with successful engraftment. Summary. Mobilisation with G-CSF + Plerixafor is an excellent strategy in the context of heavily pre-treated patients with haematological and extra haematological malignancies, can lead to early collection of HSPC and requires close collaboration between clinicians and physicians collection facilities. Further leg of this analysis will evaluate the data of patients harvested with Plerixafor + chemotherapy during neutrophil recovery and engraftment data after ASCT.

Table 1.

<table>
<thead>
<tr>
<th>Characteristics of patients and collection</th>
<th>No of Patients</th>
<th>Diagnosis (Myelodysplastic/Hodgkin/Hodgkin lymphoma/Lymphoma/Lymphoma/Chronic Lymphocytic Leukemia/Malignancies)</th>
<th>Median Weight (range) kg</th>
<th>Median sessions of apheresis (range)</th>
<th>Median Doses of G-CSF (µg/kg)</th>
<th>Median Total blood volume processed at first apheresis (range) Liters</th>
<th>Median CD34+ cells x10⁶ collected at day 1 of apheresis</th>
<th>Median CD34+ cells x10⁶ collected at day 2 of apheresis</th>
<th>Median CD34+ cells x10⁶ collected (range)</th>
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<tr>
<td></td>
<td></td>
<td>65</td>
<td>10 (7-15)</td>
<td>10 (6-18)</td>
<td>10,4 (4.4-20.6)</td>
<td>1.8 (1.0-1.65)</td>
<td>1.1 (1.0-1.46)</td>
<td>3.6 (2.6-7.7)</td>
<td>2.6 (2.6-7.7)</td>
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Background and Aims. Allogeneic stem cell transplantation (allo-SCT) is a potentially curative treatment option for patients with hematological disorders. Alloreactive donor-derived T lymphocytes exert a beneficial graft-versus-leukemia (GVL) effect through the recognition of leukemia-restricted (or preferentially expressed) antigens as Wilms tumor protein (WT1), survivin (SURV) or proteinase (PR1). Current research in transplant immunology focuses on enhancing GVL while preventing the deleterious graft-versus-host disease (GVHD) that could be achieved by manipulating donor-derived antigen-specific T-populations. In this study we sought the presence of peripheral blood leukemia-associated antigen-specific CD8+ T-lymphocytes and the impact on outcome after allo-SCT. Methods. 54 consecutive HLA-identical patients undergoing conventional myeloablative (n=22) or non-myeloablative (n=22) allo-SCT as treatment of hematological disorders were included. Donor was an HLA-identical sibling in 46 cases (80.5%) and unrelated in 8 cases (14.5%). Stem cell source included mobilized peripheral blood (n=22), bone marrow (n=25) and umbilical cord blood (n=7). Twenty-nine patients received rabies anti-hemocyte globulin at 6-8mg/kg. Wtih a median follow up was 27.5 months (range 2-88) four patients had relapsed 9-14 months after allo-SCT. We used four color multiparametric flow cytometry in a FACSCanto II acquiring at least 1 x10⁶ events (Propidium iodide low) lymphoid gated events, stained with Mabs: CD8-FITC and CD3PE/APC. To identify leukemia-antigen specific CD8 lymphocytes we used APC or PE conjugated class I HLA*0201 pentamers (Proimmune) against the following nonapeptides: Proteinase 1:VLOELNVTY, WT1:RMFPNAPYL and SURV:ELTL-GEFLKL. Functional assessment of CD8+ was performed by intracellular IFN-γ detection using 1x10⁶ PBMCs stimulated with or without SURV, PR1 and WT1 synthetic peptides (10µg). After one hour in culture 10µg/ml brefeldin A was added. As negative controls we used PE/APC labelled HLA-A*0201 negative control pentamer and as positive control we used CMVpp65/HLA-A*0201. Results. Donor-derived lymphocytes against PR1, WT1 and SURV were detected in peripheral blood samples in 56.4%, 47.5% y 37.1% of recruited patients respectively. Median percentage of anti-PR1 was 0.051% (range:0.001-0.367% over CD3+CD8+ events), 0.03% for WT1 (range:0.001-0.457%) and 0.20% for SURV (range:0.001-0.250). Detection of leukemia-antigen specific CD8+ lymphocytes was significantly associated with biological variables such as conditioning regimen (conventional or non-myeloablative), age donor, alloSCT source. The presence of anti-PR1 specific CD8+ lymphocytes was significantly more frequent in patients grafted with an HLA-identical sibling donor (P<0.01), and in patients not receiving ATG (P<0.05). In addition, the presence of circulating anti-SURV specific CD8+ lymphocytes was more frequently found in patients developing GVHD post-allo-SCT (P=0.043). Detection of circulating anti-PR1 and anti-SURV specific CD8+ lymphocytes was associated with less mortality rate (P<0.01 and P<0.01 respectively). In the univariate analysis we found that overall survival and event free survival was significantly better in patients with anti-PR1 and anti-SURV specific circulating CD8+ lymphocytes (P=0.01 and P=0.024, respectively). Conclusions. Multiparametric flow cytometry is a useful tool to detect and quantify rare donor-derived CD8+ lymphocytes specific for leukemia-associated antigens as PR1, WT1 or SURV. The presence of anti-PR1 and anti-SURV specific CD8+ lymphocytes in peripheral blood is associated with a better survival and this finding could be related to an increased immunosurveillance against residual tumor cells in allo-SCT.

0440

SEQUENTIAL STRATEGY OF REDUCED-INTENSITY ALLOGENEIC HEMATOPOIETIC STEM-CELL TRANSPLANTATION (IDA-FLAG-MELPHALAN) FOR PATIENTS WITH PRIMARY REFRactory OR RElapsed ACUTE MYELOID LEUKEMIA

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Background. The prognosis of patients with acute myeloid leukemia (AML) failing to frontline therapy remains poor, with allogeneic hematopoietic stem-cell transplantation (alloH SCT) arising as the only option with long-term curative potential. Nonetheless, only a minority of such patients ultimately undergo alloH SCT after salvage therapy. Sequential strategies are aimed to integrate a cytoreductive phase followed by reduced intensity conditioning in a sole procedure, in order to increase the proportion of patients who might benefit from the alloH SCT graft-versus-leukemia effect. Aims. To compare the outcome of a series of patients with primary refractory (Ref) or relapsed (Rel) AML treated according to a sequential protocol with a group of patients receiving conventional salvage chemotherapy in a single institution. Patients and Methods. In 2005 we designed a sequential protocol based on a cytoreductive phase with the IDA-FLAG regimen (days -11 to -7) followed by melphalan (70 mg/m² x days -3 & -2) for patients with Rel or Ref AML not considered candidates to a myeloablative alloH SCT, due to age >50 and/or previous transplantation. The outcome of these patients (SEQ arm) was compared with a cohort of Ref/Rel AML cases from our centre treated since 1998 with a standard approach (CONV arm), consisting of 1-2 cycles of IDA-FLAG followed by alloH SCT in responding patients. Results. Overall, 22 patients (age: 52, 29-65; 50% male) included in the SEQ arm were compared to 51 patients (age: 48, 22-68; 53% male) treated according to CONV strategy. Within SEQ arm, a higher proportion of patients were diagnosed of secondary AML (36 vs. 8%, P=0.005), received more lines of previous therapy (2± 4 vs. 6%, P<0.001), and had previously undergone H SCT (41 vs. 4%, P<0.001), whereas the CONV arm contained a higher proportion of Rel AML (59 vs. 27%, P=0.013). The SEQ strategy was followed by a high immediate antileukemic response (CR rate at day +30, 95 vs. 45% after CONV arm, P<0.001). Of note, only 38% of patients in CONV arm finally received an alloH SCT. Among patients achieving CR after salvage therapy, cumulative incidence of relapse at 2-yr was 31% (95% CI: 15-67%) and 45% (95% CI:29-72%) in the SEQ and CONV arms, respectively. Moreover, when patients that achieved CR after salvage therapy were considered, overall survival was significantly better (95% CI: 29-72% vs 17% respectively, P<0.001). In univariate analysis we found that overall survival and event free survival was significantly better in patients with anti-PR1 and anti-SURV specific CD8+ lymphocytes in peripheral blood associated with a better survival and this finding could be related to an increased immunosurveillance against residual tumor cells in allo-SCT.
non-relapse mortality (NRM) at 2-yr was 41% (95% CI: 24-71%) and 31% (95% CI:17-58%) after SEQ and CONV strategy, respectively. As a result, overall survival at 2-year after salvage therapy was 54±12% and 18±4% for patients treated according to SEQ strategy and CONV arm, respectively, without achieving statistical significance (p=0.12, see figure). Conclusions. Despite a remarkable antileukemic effect, the herein described sequential strategy did not result in a survival benefit over a standard salvage strategy, probably due to high non-relapse mortality. Further strategies aiming to diminish toxicity and relapse after allogeneic are warranted to improve the overall results of sequential strategies for the management of refractory and relapsed AML.

0441
HIGH DOSE THERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN RELAPSED/PRIMARY REFRACTORY HODGKIN LYMPHOMA PATIENTS: OUTCOME AND PROGNOSTIC FACTORS. EXPERIENCE FROM TWO GREEK CENTERS
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Background. High dose therapy and autologous hematopoietic stem cell transplantation (HDT/ASCT) is considered the standard of care for patients with relapsed or primary refractory Hodgkin lymphoma (HL). Aims. To analyze the prognostic factors and outcome of HDT/ASCT in 142 patients from the Department of Hematology and the 2nd Department of Propaedeutic Internal Medicine of the University of Athens were studied. Progression free survival (PFS) was calculated from ASCT to relapse/progression or death and overall survival (OS) from ASCI to death from any cause. Results. Median age at HDT/ASCT was 50 years (17-66) and 56% were male. At diagnosis, 8% had clinical stage I, II, III and IV respectively, 43% had B symptoms, 31% bulky disease, 26% were initially treated with ABVD, while 39% received additional radiotherapy. At relapse/progression 14%, 43%, 5% and 38% of the patients had clinical stage I, II, III and IV respectively, 19% B symptoms, 10% bulky disease and 41% extranodal disease. Forty-three % of them were treated with HDT/ASCT for 1st relapse, 11% for multiple relapses and 46% for primary refractory disease. After the last salvage chemotherapy and just prior to HDT/ASCT, 38% were in complete remission, 44% in partial remission and 23% were chemorefractory. At a median follow-up of 45 months (2-173), 5-year PFS was 50%, while OS at 5 and 10 years was 81% and 72% respectively. Chemoresistance before HDT/ASCT (p<0.0001), bulky disease (p<0.02) and B symptoms at relapse (p<0.002) proved to be poor prognostic factors for PFS. Thus, 5 year PFS was 56% for chemosensitive patients vs 21% for chemorefractory ones, respectively. Moreover, patients who were transplanted due to multiple relapses had a better outcome compared to others (p<0.05). Chemoresistance (p<0.0001), bulky disease (p<0.0001) and B symptoms at relapse (p<0.0003) were found statistically significant for OS, as well. In addition, age ≥ 45 years proved an unfavorable prognostic parameter for OS (p<0.01). Multivariate analysis documented the independent prognostic value of chemosensitivity and B symptoms for PFS and OS. Age was also an independent prognostic factor for OS. Conclusions. HDT/ASCT may cure half of the patients with relapsed or primary refractory HL. Chemorefractory patients and those with bulky disease and B symptoms at relapse or progression have a dismal outcome.

0442
AUTOLOGOUS STEM CELL TRANSPLANTATION AS A CONSOLIDATION TREATMENT FOR MANTLE CELL LYMPHOMA
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Background. Mantle cell lymphoma (MCL) is an aggressive subtype of B-cell non-Hodgkin lymphoma with poor prognosis and a reported median overall survival (OS) of 3 to 6 years. In an attempt to improve the prognosis of these patients, several therapeutic strategies have been tested, before and after introduction of monoclonal antibodies but there is no consensus about the choice first line therapy. However, a consistent number of studies show that high-dose chemotherapy followed by autologous stem cell transplantation (ASCT) increases relapse free survival (RFS) of MCL patients. Aims. To analyse retrospectively the role of ASCT (with BEAM conditioning) as consolidation for newly diagnosed MCL patients. Materials and Methods. We analysed 23 patients with confirmed diagnosis of MCL that have been submitted to ASCT between 1999 and 2010 in our institution. We included patients treated with different induction chemotherapy regimens. We collected data related to sex, age, stage of the disease, response to induction chemotherapy, evaluation at day +100 after transplant and RFS and OS were calculated. Results. Male sex was predominant (82.6%), median age was 58 years (42-67). All patients were in advanced stage disease: 91.3% in stage IV and 78.3% with leukemic expression. Eight patients were treated upfront with CHOP+/-R (x6) and 13 with Hiper-C-VAD+/R (x4). One patient was treated with R-FC and another with FCM followed by 6x R-CHOP. Fifteen patients (65.2%) attained complete remission (CR) after the induction courses in which are included 12 of 13 patients treated with Hiper-C-VAD; eight patients only achieved partial response after induction but one of these obtained a CR after R-ESHAP. In total, 16 patients (69.6%) were in CR at the time of transplant. CR rate at day +100 after ASCT increased to 86.9%. The median RFS was 58 months and the median OS was 79 months. With a median time of follow-up of 3 years and 7 months, there are 71.4% patients alive. Of these patients, 2 have relapsed so far. There was not any transplant related mortality (TRM) in this series. Conclusions. Our retrospective analysis suggests that ASCT as a consolidation therapy in MCL patients is a safe, effective and well tolerated therapeutic option, in patients under 65 years. In our study, median OS and RFS of patients that were submitted to ASCT was not reached yet. Until the last follow-up, only 1 patient treated with R-Hyper-C-VAD followed by ASCT relapsed. These results emphasize a role for Hyper-C-VAD induction followed by ASCT as frontline management of MCL patients.
Stem cell transplantation - Experimental & clinical

| 0443 |

PALONOSETRON + APREPITANT VERSUS GRANISETRON FOR PREVENTION OF NAUSEA AND VOMITING IN PATIENTS RECEIVING HIGH DOSE CONDITIONING CHEMOTHERAPY REGIMENS PRIOR TO STEM CELL TRANSPLANTATION (HSCT)

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Background. Nausea and vomiting (CINV) in patients receiving high-dose, multiday conditioning regimens prior to stem cell transplantation is particularly common (55-100% of patients) and troublesome, especially in the delayed phase (beginning 24 hours after the start of conditioning). Newer antiemetics (palonosetron and aprepitant) appear to significantly reduce acute and delayed CINV as compared to the classic serotonin antagonists; however, few studies have prospectively evaluated the efficacy of these drugs in this challenging setting. Aims. To assess whether palonosetron (0.25 mg iv every 48 h) administered in combination with aprepitant (125 mg on day 1 followed by 80 mg each of the remaining days) during the conditioning period (duration 5-6 days) improved control of CINV compared with daily granisetron (3 mg iv). Methods. This was a prospective, multicenter, randomized, stratified (by conditioning regimen), double-blind study. Patients received either BEAM (78%), BUCY (17%), or CBV/Cy/TBI (5%) as the conditioning regimen prior to HSCT. The primary efficacy endpoint was complete response (defined as no emesis and no use of rescue medication); secondary end-points included evaluation of emesis and nausea throughout the conditioning period. Adverse events were also assessed. Results. Sixty consenting patients were included in the study (n = 31 palonosetron + aprepitant; n = 29 granisetron). The mean age was 39.5 ± 20 years; 50% were women; 55% were being treated for non-Hodgkin lymphoma, and 27% for E. Hodgkin’s and 12% for AML. 94% of HSCT were autologous. There were no between-group differences in variables that could potentially influence emesis (sex, previous chemotherapy or CINV, and regular intake of alcohol). Significantly more patients in the palonosetron + aprepitant group versus the granisetron group had a complete response during the acute (0-24h; 92.5% vs 67.9%, respectively), delayed (24-120h; 61.5% vs 28.6%) and overall (0-120h; 61.5% vs 28.6%) periods. In addition, palonosetron + aprepitant significantly reduced the proportion of patients with emesis during the acute, delayed and overall periods and showed a trend toward a reduction in percent of patients with significant nausea during the delayed period (see Table). There were no significant differences between the groups in adverse events or in the times of graft infection / severe infections.

Conclusion. The combination of palonosetron + aprepitant was well tolerated with superior protection from nausea and vomiting compared with granisetron in patients receiving multiday highly emetogenic conditioning chemotherapy regimens prior to HSCT.

| 0444 |

PROGNOSTIC IMPACT OF PRE-TRANSPLANT SERUM HEPcidIN LEVELS ON CLINICAL OUTCOMES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Iron overload is an adverse prognostic factor in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT). Hepcidin, a peptide hormone produced by the liver, plays a central role in the regulation of iron homeostasis. We previously reported that elevated pre-transplant serum hepcidin levels were significantly associated with a higher incidence of early bacterial infection independent of ferritin levels (Kanda et al., BBMT 2009). Aim. To investigate the association between pre-transplant serum hepcidin levels and clinical outcomes of allo-SCT. Methods. We retrospectively studied 100 patients (age, 17-64 years; median age, 48.5 years) who underwent their first allo-SCT for hematologic malignancies at our institution between July 2006 and December 2010. Written informed consent was obtained from all patients. The primary diseases were myeloid (n = 66) and lymphoid malignancies (n = 34). Serum hepcidin-25 levels prior to the administration of conditioning regimen were measured using a liquid chromatography-tandem mass spectrometry-based assay system. The primary endpoint was overall survival after allo-SCT, and the secondary endpoints were the cumulative incidence of acute and chronic graft-versus-host disease (GVHD), transplant-related mortality (TRM), and relapse. Factors evaluated in the analysis included the recipient’s age, sex, diagnosis, disease status, source of stem cells, conditioning regimen, GVHD prophylaxis, serum hepcidin levels, ferritin levels, and C-reactive protein (CRP) levels. Results. The median hepcidin level of the patients was 29.3 ng/mL (range, 0.4-371 ng/mL; normal level, 22.2 ± 12.3 ng/mL). There was a weak correlation between hepcidin and ferritin levels (r = 0.217, P = 0.030). These patients were divided into 2 groups: the low-hepcidin group (<30 ng/mL, n = 50) and the high-hepcidin group (>30 ng/mL, n = 50). Ferritin levels were higher in the low-hepcidin group (49% vs. 69%, P = 0.078). The incidences of chronic GVHD, TRM, and relapse were not different between the 2 groups. Conclusions. In the present study, elevated pre-transplant hepcidin levels were associated...
with an inferior overall survival in the univariate analysis, but not in the multivariate analysis. Our data confirmed that elevated ferritin and CRP levels were strong adverse prognostic factors for survival, as many other studies have shown. In addition, we observed a tendency toward a higher incidence of grade 3-4 acute GVHD in the high-hepcidin group. Larger studies are necessary to elucidate the association between hepcidin levels and acute GVHD.

0445
EXTRACORPOREAL PHOTOPHERESIS FOR STEROID REFRACTORY OR DEPENDENT ACUTE GVHD IN PEDIATRIC PATIENTS

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Background. Acute graft-versus-host disease (aGVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Glucocorticoids are the standard treatment for aGVHD. Patients who fail to respond to this first-line therapy have a poor prognosis, with high transplant-related mortality due to GVHD itself and to its treatment complications such as opportunistic infections. Extracorporeal photopheresis (ECP) has been shown to be effective for patients with aGVHD not responsive to conventional therapeutic approaches. We report our experience on ECP treatment in pediatric patients affected by aGVHD. Aims. To evaluate the efficacy of extracorporeal photopheresis for the treatment of steroid-refractory or steroid-dependent aGVHD in pediatric patients. Methods. From 1997 to 2009, 49 children (52 males, 17 females) with steroid-refractory or dependent aGVHD have been treated with ECP. Patients underwent HSCT for ALL (n=25), AML (n=15), NHL (n=4), CML (n=5) or other non-malignant diseases (n=2). The median age at ECP was 8.7 years (range: 0.95-18.7) and the median body weight was 29 Kg (range: 7-98). The stem cell sources were unrelated donors (n=32), siblings (n=11), cord blood (n=5) or haploidentical donor (n=1). The overall clinical stage of aGVHD was grade II (n= 24), grade III (n=13) and grade IV (n= 12). Cutaneous GVHD was diagnosed in 44 children, liver and gastro-intestinal tract involvement in 11 and in 43 children respectively. ECP was started after a median interval from HSCT of 42.5 days (range: 13-91) and after a median time from aGVHD onset of 28.5 days (range: 4-91). A Hickman-Browec double-lumen central venous line was used in all patients. The median duration of treatment was 4.6 months (range: 0.5-10.2), with a median number of 10 cycles (range: 3-45). ECP was performed using the on-line technique (n=17) or using off-line technique (n=32). Results. 34 patients (69.4%) survived while 15 died (30.6%), due to relapse of the underlying disease (n=9), GVHD (n=3), CMV-related interstitial pneumonia (n=2) or encephalopathy (n=1). Among the 34 patients who survived, a complete, partial, or no response to ECP was seen in 79%, 0%, and 21% of patients with aGVHD II respectively, in 69%, 15% and 16% of patients with aGVHD III, and in 50%, 38% and 17% of those with GVHD IV respectively. Complete response of aGVHD manifestations of skin, gut and liver was observed in 75%, 74%, 91% of patients respectively. Conclusions. Our results confirm the efficacy of ECP in the treatment of steroid resistant or dependent aGVHD in pediatric patients. Moreover, for all patients, cutaneous aGVHD, gut and liver, a good response to ECP was found.

0446
SIBLING CORD BLOOD TRANSPLANTATION FOR DIAMOND-BLACKFAN ANEMIA: THE EFFECT OF MINOR HISTOCOMPATIBILITY ANTIGENS ON GVHD

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Diamond-Blackfan anemia (DBA) is a clinically and genetically heterogeneous condition characterized by pro-apoptotic hematopoiesis, congenital anomalies and predisposition to cancer. Cord blood (CB) stem cell transplantation is becoming an effective cure for DBA patients, especially if HLA-identical sibling donors are available. In transplantation setting, in addition to Major Histocompatibility Complex antigens (HLA), minor Histocompatibility Antigens (mHAg) may affect the outcome in terms of engraftment, rejection and Graft versus Host Disease (GVHD). In HLA-identical sibling transplantation, mHAg mismatches can stimulate T-cell activation, leading to specific alloreactivity against donor’s or recipient’s antigens, consequently supporting rejection or GVHD, respectively. We considered four DBA patients and their HLA-identical (HLA-A,-B,-DRB1) CB sibling donors to investigate the role of 12 polymorphic mHAg: HA-1, HA-2, HA-3, HA-8, HB-1, ACC-1, ACC-2, CDS1 (codons 125, 568 and 670), CD62L (codons 206 and 213), PANCE-1, UTC2187, SP110 (mHAg Ministry kit, University Clinic Heidelberg, Germany), and HY. The patients’ characteristics and the cell dose of CB grafts are shown in the table. All patients reached full chimerism, and no-one experienced graft failure. At present, they are all alive and disease-free. Considering the two directions of mHAg mismatches (GvHD: donor versus recipient; rejection: recipient versus donor), we investigated the mHAg-mismatching-grade in all patients with respect to GVHD, neutrophil and platelet recovery times. The only patient (D) who experienced aGvHD showed no mHAg mismatches in GVHD direction, while the other ones, with no signs of aGvHD, showed at least three mismatches in GVHD direction (see the graph). Moreover, going from patient A to patient D, the mHAg-mismatching-grade increases in rejection direction, as well as platelet recovery time augments (21 to 44 days), whereas the mHAg-mismatching-grade in GVHD direction decreases. Despite this kind of sample (homogeneous for HLA-identity, non-malignant condition, conditioning regimen and GVHD prophylaxis) could be suitable to investigate the mHAg effect on CB transplantation, its size is restricted. However, we put forward a few hypotheses. Data regarding patient D seem to be in contradiction with the classical histocompatibility assumption, which predicts GVHD according to HLA disparities in GVHD direction. Moreover, patient D received Treosulfan-based conditioning regimen, which is reported to be less toxic than Busulfan. At most, Treosulfan may help to overcome the mHAg-mismatching-grade in rejection direction, as it is associated to excellent engraftment results. Therefore, trying to explain the occurrence of aGVHD in patient D, the involvement of an autologous-GvHD syndrome could be reasonably hypothesized. Recent studies in human and animal models highlighted that an autoimmune syndrome can occur after bone marrow transplantation (BMT) between identical twins, or even after autologous BMT, and this syndrome seems to be pathologically identical to the GVHD after allogeneic BMT. Autologous-GvHD is a special autoaggression syndrome which may cause the exacerbation of GVHD. Two major factors may induct an autologous-GvHD: the failure to re-establish peripheral self-tolerance and the disruption of thymic-de...
pendent immune reconstitution. Further investigations are needed to confirm the role of mHAGs on GVHD, first of all by enrolling in the study an increased number of patients.

**0447**

**EFFICACY OF MOBILIZATION WITH PLERIXAFOR IN PATIENTS FAILING A PREVIOUS MOBILIZATION ATTEMPT AND AS FIRST-LINE MOBILIZING THERAPY IN PATIENTS AFFECTED BY MULTIPLE MYELOMA**

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Peripheral blood stem cells (PBSC) represent a well established viable alternative to bone marrow as a source of hematopoietic stem and progenitor cells for autologous transplantation, a standard approach for treatment of different hematological malignancies. A dose of 2.0x10^6 CD34+cells/kg is generally considered sufficient to allow a successful engraftment after high-dose chemotherapy. Nonetheless a high fraction of patients (pts) ranging from 11 to 53% will fail mobilization of stem cells and won’t be able to benefit from autologous stem cell transplantation (ASCT). Plerixafor (AMD 3100) a bicyclam antagonist of the SDF-1 alpha/CXCR4 complex, has been previously reported to improve PBSC collection in pts undergoing PBSC mobilization. From April 2009 to February 2010, a total of 13 patients affected by hematological malignancies (5 Hodgkin Lymphoma, 5 Non-Hodgking Lymphoma, 3 Multiple Myeloma) who had already failed a previous mobilizing attempt, underwent stimulation with plerixafor at a standard dose after receiving G-CSF for 4 days in order to mobilize PBSC; other 6 patients (pts) who have never been mobilized before, received the same stimulation therapy following immunomodulatory drugs containing induction chemotherapy for multiple myeloma (MM). Pts characteristics were the following: 15 were female, 4 were male, median age was 53 years (27-70); median number of previous lines of therapy was 2 (1-7) and 4 pts had received radiotherapy. Overall plerixafor administration was safe and no serious adverse events were reported. The median number of circulating CD34+ cells/ul following plerixafor was 22 (11-138). All 19 patients were able to collect the minimum required dose for ASCT in a median number of procedures of 1 (1-3); median numbers of CD34+ cells collected was 2.5x10^6/kg; notably the 6 patients affected by MM, stimulated with plerixafor upfront were able to collect in a single procedure the target CD34+ dose to be used for a tandem transplant. At the time of the analysis, 13 of the 19 pts had already undergone ASCT: 13/13 engrafted with a median time to ANC 500/ul of 12 days and to a PLT ≥20000 of 16 days. We conclude that mobilization with plerixafor is safe and effective being able to rescue patients who failed a previous mobilizing attempt and likely being an effective mobilization strategy for pts with MM who need to collect a greater number of CD34+ cells/kg for a tandem transplant.

**0448**

**MOYOABLATIVE CONDITIONING FOLLOWED BY UNMANIPULATED HAPLO-MISMATCHED MARROW FOR ADVANCED HEMATOLOGIC MALIGNANCIES**

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Background. Haplomismatched marrow has been grafted successfully following a non myeloablative conditioning (NMA) and high dose cyclophosphamide (HDcy) post-transplant. HDcy is capable of preventing acute graft versus host disease (GvHD), despite the HLA mismatched haploptotype (Luznicki et at, BBMT 2008) Aim of the study. In this study we tested whether HDcy could also prevent aGvHD in a setting of myeloablative conditioning regimen. Methods. Patients were prepared with Fludarabine 50 mg/m^2/2/day x5, i.v. Busulfan 3.2 mg/kg/day x5, Thiopeta 5 mg/kg/dayx2. HDcy was given at 50 mg/kg day+3, day+4 and given in two divided doses given from -2 to -1. One patient was prepared with FLU-TBI. Marrow was harvested from haplo-mismatched family members according to standard procedures. Results. We have grafted 16 patients with leukemia beyond second remission: the diagnosis was AML (n=4), ALL (n=2), CML (n=2), lymphoma (n=3), myelofibrosis (n=1), myelodysplasia (n=3). Three patients were receiving a second allogeneic transplant. The median marrow cell dose given was 4.2x10^8/kg (range 2.7-7.8). Median day to 0.5x10^9/L neutrophils was day+20 (15-30). Two patients died of hemorrhage before day 7. Of the 14 evaluable patients all engrafted with 100% donor chimerism by day +30. Hematologic recovery was complete in all patients. GVHD was scored as grade I in 7 patients and grade II in one patient. No patients developed grade III-IV GVHD: CMV and EBV infections were not a problem in this initial series of patients. Transplant related mortality was seen in 4 patients (25%). 3 patients died of leukemia relapse. 11/16 patients survive (68%) 30-280 days post transplant. Conclusions. This initial series of patients suggests that (a) haplo mismatched unmanipulated marrow can be grafted after myeloablative conditioning; (b) hematopoietic recovery is reliable and complete (c) transplant mortality acceptable for a group of advanced patients and (d) most importantly transplants can be organized in useful time for advanced leukemia.

**0449**

**HIGH BASELINE BAALC EXPRESSION AT DIAGNOSIS PREDICTS POOR OUTCOME IN PATIENTS WITH MULTIPLE MYELOMA FOLLOWING AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION**

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Background. High BAALC transcripts are associated with unfavorable outcome in newly diagnosed acute myeloid leukemia (AML) patients. **Aims.** We analyzed BAALC gene expression in patients with core binding factor (CBF) positive AML undergoing hematopoietic stem cell transplantation (HSCT) in order to assess whether this prognostic impact parallels with the post-transplantation outcome. **Methods.** Among fifty three consecutive adult patients diagnosed as CBF-AML who were transplanted from January 2008 and June 2010 in Catholic Blood and Marrow Transplantation Center, quantification of BAALC transcripts by real-time quantitative PCR in diagnostic bone marrow (BM) was performed. **Results.** We retrospectively analyzed the treatment outcome on 31 CBF-AML patients who completed induction chemotherapy and underwent stem cell transplantation. **Results.** The median age at diagnosis was 45 years (range, 16-61). The median number of BAALC transcripts measured on diagnostic BM of available 41 samples were 62.293 (range, 9.587 - 497.4). All patients (n=31) achieved complete remission (CR) after 1 cycle of induction chemotherapy and remained so by the time of transplantation. The median BAALC transcript level after achieving CR reduced to 2.171 (range, 0.251-31.604). At the time of transplantation after 1 or 2 courses of consolidation chemotherapy, the median transcript level was 1.1705 (range, 0.291-54.174). Six patients (19%) had complex karyotype and c-kit mutation was detected in 11 patients while NPM in two patients. FLT3 was negative in all patients. Thirteen patients (42%) received autologous stem cell transplantation while the remaining 18 patients underwent allogeneic HSCT; myeloablative and reduced intensity conditioning in 14 and 4 patients, respectively. The median follow-up duration of survivors was 12.25 months (range, 1.54-25.13). The estimated 1-year overall survival and disease-free survival of all patients were 68.7% (±10) and 65.9% (±10), respectively. The 1-year cumulative incidence of treatment related mortality was 15.34% (±7.3) while that of relapse was 10.55% (±5.8). When the baseline BAALC transcript levels were dichotomized into high and low levels, as high level being upper quartile, the higher level group had significantly poor results in 1-year cumulative incidence of relapse (4.3 % vs 37.5%, p-value=0.012). **Conclusions.** CBF-AML patients expressing high BAALC RNA levels at diagnosis had significantly higher risk of relapse after undergoing transplantation.
Aims. We here report the clinical outcomes of patients with MM allo-
grafted at the Geneva University Hospital between 1988 and 2010. Methods: 23 patients (15 males and 7 females) were included in this ret-
rospective analysis. Their median age at transplantation was 48 (range, 24-75) years and the median interval from diagnosis to allo-HSCT was 18 (range, 5-78) months. Seventy (70%) patients had IgG, 3 (13%) IgD, 1 (4%) IgA and 3 (13%) light chains MM. Almost all patients (91%) were conditioned with BEAM or BEAC. Areas not in CR prior to transplantation; pretransplant cytoreductive therapy varied, but all were conditioned using BEAM or BEAC. Areas not in CR prior to transplantation were irradiated after hematological recovery. Results. Thirty-one were refractory, 26 in early relapse, 25 in late relapse, 9 in relapse of unknown duration, and 5 patients had nodular sclerosis, 22 mixed cellularity, 2 lymphocyte predominance HL and in 3 the type was unknown. With a median follow-up of 34 months 3-year and 5-year overall survival (OS) of the entire cohort are 78% and 66% and event-free survival (EFS) 66% and 60% respectively (Fig). HL type, response to last previous treatment (refrac-
tory vs. early relapse vs. late relapse vs. multiple relapses), pretransplant cytoreductive therapy varied, but all were conditioned using BEAM or BEAC. Areas not in CR prior to transplantation were irradiated after hematological recovery. Results. During this period 87 patients, 52 men and 35 women, 15-55 years old (median 30) with relapsed / refractory HL were autografted at our institution. Thirty-one were refractory, 26 in early relapse, 25 in late relapse and 5 had multiple relapses prior to transplantation. Sixty pa-
patients had nodular sclerosis, 22 mixed cellularity, 2 lymphocyte predomi-
nant HL and in 3 the type was unknown. With a median follow-up of 34 months 3-year and 5-year overall survival (OS) of the entire cohort are 78% and 66% and event-free survival (EFS) 66% and 60% respectively (Fig). HL type, response to last previous treatment (refrac-
tory vs. early relapse vs. late relapse vs. multiple relapses), pretransplant therapy (miniBEAM vs. DHAP vs. high-dose ifosfamide and mi-
oped HHV-6 encephalitis. Examination of plasma cytokines concentra-
tions showed that peak concentrations of IL-6, IL-10, IL-13, MCP-
1, and MIP1beta were significantly higher in patients who developed HHV-6 encephalitis than in patients who did not. In particular, IL-6 concentration was very high in patients who developed HHV-6 en-
cephalitis (median, 1215 pg/ml versus 70.1 pg/ml, P = 0.0007). None of the 88 patients whose peak IL-6 concentration was less than double the median (155 pg/ml) developed HHV-6 encephalitis, while 6 of 32 pa-
tients (19%) with peak IL-6 concentration more than double the me-
dian developed HHV-6 encephalitis. Among the 6 patients who devel-
oped HHV-6 encephalitis, central nervous system dysfunction devel-
oped concomitant to peak HHV-6 DNA in each patient. Plasma IL-6 con-
centration peaked 1 week before the development of HHV-6 en-
cephalitis in 5 patients and at the time of developing HHV-6 en-
cephalitis in 1 patient. Discussion. Increased IL-6 before the develop-
ment of HHV-6 encephalitis suggests the involvement of increased IL-
6 production in the pathogenesis of HHV-6 encephalitis. Control of in-
flammatory conditions before engraftment may prevent the develop-
ment of HHV-6 encephalitis.
toxantrone) and date of transplantation did not affect outcome. Older age at transplantation was a negative prognostic factor for OS but not EFS. The only statistically significant prognostic factor that we were able to identify was response to pretransplant cytoreductive therapy. In the group transplanted in CR, 3-year OS was 87% and EFS 85%, in PR 80% and 63%, in stable disease 52% and 20% and in progressive disease 28% and 25% respectively. This difference is highly statistically significant (p<0.001, log-rank test). Conclusions. Outcome of autografted HL patients has not changed significantly in the last 15 years. Sixty percent of these patients remain long-term free of their cancer. In our experience, response to pretransplant cytoreductive chemotherapy is a more important prognostic factor than response to last previous treatment.

**0453**

**GASTROINTESTINAL SYMPTOMS FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION: MYCOPHENOLEATE MOFETIL OR GRAFT-VERSUS-HOST-DISEASE?**

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**Background.** Gastrointestinal (GI) complications occur frequently after allogeneic stem cell transplantation (alloSCT). Difficulties in confirming a diagnosis could emerge when mycophenolate mofetil (MMF) is used as part of the GVHD prophylaxis regimen, as it may cause gastrointestinal toxicity mimicking graft-versus-host-disease (GVHD). Currently, dose reduction or even discontinuation of MMF is the only way to clarify the origin of gastrointestinal symptoms, though this could be dangerous if GVHD is the cause of the symptoms. Furthermore, there may be an increased risk of graft rejection associated with premature discontinuation of MMF. Aim. The aim of this study is finding markers that enable to differentiate between gastrointestinal symptoms as caused either by MMF or by GVHD. This would prevent unnecessary discontinuation of MMF in patients that suffer from GI GVHD and would better guide physicians in establishing which patients may require MMF discontinuation. Methods. All stored gastric and colonic biopsy specimens at the department of clinical pathology, that were taken between 2004 and 2009, of patients who suffered from GI symptoms and had received MMF after allogeneic SCT were reviewed. Additionally, clinical data were retrieved by review of medical records. Biopsies were scored for apoptosis, crypt cell destruction, inflammation of the lamina propria and denudation of the mucosal wall. Together these parameters constituted the histological grade of gastrointestinal damage. Clinical features contained clinical grade of GVHD, temporal relations between start of MMF and occurrence of symptoms, the effect of interruption of MMF and the final clinical diagnosis. Results. Biopsies of 54 patients were available. In 16 patients (29.6%) the gastrointestinal symptoms were assigned to MMF, in 34 patients (63%) to GVHD and in four patients (7.4%) to infection. Based on a 7-level scale scoring epithelial apoptosis (no apoptosis=0, none-minor=1, minor=2, minor-intermediate=3, intermediate=4, minor-severe=5, intermediate-severe=6 and severe=7) the mean score (SD) in the MMF group was 3.31 (1.78) and in the GVHD group 4.47 (1.88). This difference was statistically significant (p=0.047). No other significant differences were revealed by review of the histological specimens. Summary/conclusions. In almost 50% of patients who had histological evidence of gastrointestinal damage, the symptoms were designated as MMF-induced. Histological differentiation between gastrointestinal toxicity due to either MMF or GVHD may be supported by scoring the grade of epithelial apoptosis, which could therefore be important in deciding whether to stop MMF or not.

**0454**

**BK-RELATED HAEMORRAGHIC CYSTITIS IN ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A SINGLE CENTER EXPERIENCE**

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**Background.** Haemorrhagic cystitis (HC) is considered a primary manifestation of BK virus (BKV) infection in patients undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT). Roughly 50-100% of all allo-SCT recipients develop BK viruria, while only 5-40% progress to HC, which is associated with significant morbidity and mortality. Aims. To evaluate the incidence, associated risk factors and clinical impact of HC in a single centre series during a three-year period (2008-2010). Methods. All consecutive allo-SCT performed at our unit during the period of study were evaluated prospectively for HC. All patients received the same graft-versus-host-disease (GVHD) prophylaxis. BK viruria and BK virus load in plasma were systematically recorded, along with a set of clinical variables: age, sex, underlying diagnosis, type of donor, source of stem cells, conditioning regimen, presence of symptoms, grade of GVHD, HIV status and absolute neutrophil count. Samples of urine and plasma were tested for BKV by PCR. Kaplan-Meier method was used for survival analysis, and log-rank test for differences in overall survival (OS). Statistical analysis were performed with SPSS v 15.0 package. Results. 57 patients had an allo-SCT during the period of study, four of them were HIV positive. The median age was 41 (17-68), 24 men (64.9 %) and 13 women (35.1 %). 13 patients (21.4 %) developed HC between 4 and 115 days after allo-SCT: 10 of 24 men (41.6 %) and 3 of 13 women (23 %); only one of the 13 HC patients was HIV positive; 3 of 5 (60%) had grade ≥3 and 10 of 32 (31.2%) ≤ 2 GVHD; 3 of 14 (21.4 %) and 10 of 23 (43.5 %) had a related or unrelated donor, respectively. 4 patients had only dysuria while 20 patients had no genitourinary symptoms. A specific BK viruria presented in 10/20 (50 %) in asymptomatic group, 3 of 4 (75 %) in group of dysuria and 12 of 15 (92.3 %) in the HC group. Median survival (see figure 1) was 12.3 months (2.7-21.8) and 8.2 (1.9-14.5) in the non-HC and HC groups, respectively (p=0.113). Conclusions. HC is a serious and frequent event in the allo-SCT setting. We confirm a tendency to HC in patients with unrelated donors and severe GVHD. Every effort should be made to minimize this complication in order to improve transplant-related mortality.

**0455**

**IMPACT OF INFAMMATORY CYTOKINE GENE POLYMORPHISMS ON DEVELOPING ACUTE GRAFT VERSUS HOST DISEASE IN CHILDREN RECEIVING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background.** Acute Graft Versus Host Disease (aGVHD) is one of the major causes of morbidity and mortality in the first 100 days after Allogeneic Hematopoietic Stem Cell Transplantation (HSCT). It can occur, despite aggressive immunosuppressive prophylaxis, even when the donor is a “perfectly”matched (HLA-identical) sibling, suggesting that the risk of developing this complication does not only depend on HLA matching. Many recent studies have reported the association between inflammatory cytokine gene polymorphisms and the occurrence of aGVHD in transplanted adults, but few data are available about the pediatric population. Aims. The aim of this study is to analyze the association between a wide panel of inflammatory gene polymorphisms and the occurrence of aGVHD in a setting of pediatric HSCT from HLA-
matched family and unrelated donors (MFD and MUD). Methods. The study population consisted of 156 children who underwent HSCT at the Pediatric Hematology-Oncology of the Bologna University between 1995 and 2010 and their respective donors. Patient (pts) and donors characteristics are resumed in Table 1. Genotypes of 38 single nucleotide polymorphisms (SNPs) in 19 immunoregulatory genes known to be related to GVHD onset risk (ESR1, FAS, FCGR2A, IL1A, IL1B, IL2, IL6, IL10, IL10RB, IL18, MBL2, MTHFR, NOD2, TGFB1, TGFBR2, TLR4, TNF, TNFRSF1B and VDR) were determined by the Sequenom MassARRAY system. Results were checked for Hardy Weinberg equilibrium and filtered to exclude SNPs with minor allele frequency < 0.15. Association with aGVHD was analysed in a case-control study format by Chi-square statistics, with OR ±95%CI estimation, using SNPator package (http://www.snpator.org). Results. aGVHD (grade I-IV) was observed in 17/44 (38%) pts and 53/92 (57%) receiving a MFD and a MUD respectively. In the whole cohort of pts we found statistically significant association with aGVHD grade I-IV (P=0.01, OR 3.03, CI: 1.25-7.36; P=0.01, OR 2.87, CI: 1.23-6.66, respectively). Conclusions. Polymorphisms in genes of clear importance in the regulation of the inflammatory response such as TNF, IL1A, IL1B and IL10 play a crucial role in the risk of developing aGVHD. In particular the IL10 gene SNPs were associated with aGVHD grade I-IV (P=0.04, OR 2.76, CI: 1.03-7.40). Regarding the MUD cohort, a statistically significant association with grade I-IV aGVHD was observed for donor IL1B 8954AT and donor and recipient TNF+488C and -857C (P=0.01, OR 3.03, CI: 1.25-7.36; P=0.003, OR 3.29, CI: 1.45-7.49, P=0.01, OR 2.87, CI: 1.23-6.66, respectively). Background. Chronic Graft versus host disease (cGVHD) remains a major cause of morbidity and mortality following allogeneic stem cell transplantation (SCT), affects up to 60% of patients who survive 100 days post transplant and significantly impacts upon quality of life. Novel therapies for the treatment of cGVHD are required. The chemokine-receptor axis represents an important mechanism by which lymphocytes traffic to target organs and we therefore wished to explore this pathway in the pathogenesis of chronic GVHD. Aims. The study aimed to identify key chemokines associated with the pathogenesis of tissue-specific cGVHD post allogeneic SCT and to determine whether they represent potential therapeutic targets. Methods. Patients were recruited onto the study following informed consent prior to undergoing allogeneic SCT. Peripheral blood samples were taken both pre and post transplantation. Additional blood and fresh skin biopsy samples were also received at the time of skin cGVHD. A panel of cytokines and chemokines were analysed by luminescence technology in the serum of patients at the time of chronic disease (n=18), and compared to those who did not develop clinical evidence of chronic GVHD (n=3). Equal proportions of patients who underwent sibling and MUD allografts, and who underwent myeloablative and nonmyeloablative allografts were included. Patients included in the cohort had evidence of cGVHD of tissues including the skin, gut, liver, oral and ocular mucosa and the lungs. In addition chemokine receptor expression was then assessed in both the peripheral blood and tissues using 9 colour flow cytometry and immunohistochemistry. Results. The CXCR3 specific chemokines CXCL9, 10 and 11 were found to be significantly associated with chronic GVHD of the oral mucosa, skin, and eye, and with pulmonary cGVHD respectively, being elevated in the serum of patients at the time of cGVHD. In particular CXCL10 was elevated from approximately 170pg/ml to 550pg/ml in both skin and ocular disease (p<0.05). CD4+ CXCR3+ T cells were reduced in the peripheral blood of patients with skin disease from approximately 23% of CD4+ T cells in control patients to just 5% in patients with skin cGVHD (p=0.02, n=5). Levels of both CD4+ and CXCR3+ T cells were also elevated in the skin of patients with cGVHD with CD4+ cells being increased from approximately 16% (n=7) of the T cell population to 28% (n=8) and CXCR3+ cells from 50% to 63% of the T cell population. The nature of conditioning and transplant did not appear to affect the levels of CXCL10 in the serum of patients post 100 days post SCT, and regulatory T cell populations in the peripheral blood expressing IL10 play a crucial role in the risk of developing aGVHD.
CXCX3 were not significantly reduced. Summary/Conclusions. The data suggests that CXCL5-11 are associated with cGVHD, and that elevated levels of CXCL10 may result in the migration of effector T cell populations from the blood to the skin where they can cause tissue damage. CXCL10 thus represents a specific chemokine which could be targeted to both prevent or treat the symptoms of cGVHD of the skin.

**0457**

**GENETIC VARIABILITY AT LOCI CONTROLLING GLUTATHIONE HOMEOSTASIS AFFECTS TRANSPLANT RELATED MORTALITY AND SURVIVAL IN PATIENTS RECEIVING AN ALLOGENEIC HSCT AFTER A BUSULFAN-BASED CONDITIONING REGIMEN**

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Background. Busulfan is the most widely used drug for allogeneic conditioning regimens. Busulfan metabolism depends on liver glutathione (GSH) availability. A number of loci controls liver GSH synthesis and consumption, occurring during drug conjugation and oxidation process. The aim of this study was to assess the impact of genetic variability at loci controlling GSH balancing may affect allogeneic HSCT outcome. We investigated GST-1 promoter (rs7970208) polymorphism in a group of 185 patients belonging to controlled clinical trials with the aim to predict toxicity after allogeneic HSCT. The GST-1 promoter negative T cell sub-populations expand under the selective pressure of the anti-cDS2 antibody alemtuzumab. These T cells persist for years by homeostatic proliferation. In those patients, naive T cells were cGVHD positive. Since cGVHD and allemtuzumab-mediated T cell depletion in the context of allogeneic HSCT. The GST-1 promoter negative T cells present in the blood of patients after allogeneic HSCT were responsible for higher cGVHD incidence. These gene variants could be used as predictors of development of cGVHD and inflammatory complications after allogeneic HSCT.

**0458**

**GPI-ANCHOR NEGATIVE MEMORY T CELLS IN PATIENTS AFTER ALEMTUZUMAB-MEDIATED T-CELL DEPLETION HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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The anti-CD52 antibody is frequently used in allogeneic hematopoietic stem cell transplantation (HSCT) for in vivo T cell depletion (TCD). We have recently shown that CD52 negative donor T cells reconstitute in patients after HSCT following alemtuzumab-mediated TCD. T cells persist for years in the peripheral blood. We have also demonstrated that the application of donor lymphocyte infusion (DLI) has the capacity to replenish the CD52-positive T cell compartment. By staining with a fluorescent acrylamide, we have demonstrated that the lack of CD52 expression originates from a loss of glycosyl-phosphatidyl-inositol (GPI) anchors in the T-cell membrane. GPI-anchor negative T cells revealed an altered antigen specific function compared to GPI-anchor positive T cells. They secreted less IFN gamma in response to CMV peptides and allogeneic stimuli. They also showed a decreased lytic response and proliferative capacity. We further stained peripheral blood T-cells from 12 patients of different age with the surface markers CD45RA, CD45RO, CD62L and CCR7 to differentiate memory and naive CD4 and CD8 T cells. Early after transplantation, the CD4 cells were only of recipient origin and the CD8 T cells were composed of a mixture of graft-derived memory T cells. The expression of CD45RO, CD62L or CCR7 did not differ between GPI-anchor positive and negative T cell subpopulations. In many patients, we detected CD45RA-positive naive T cells later after transplantation even though the time of reconstituting naive T cells strongly differed between individual patients. The newly reconstituting naive T cells were always GPI-anchor positive. At the same time, GPI-anchor negative cells were still present among memory T cells. Our data promote the hypothesis, that graft derived GPI-anchor negative memory T cells persist and proliferate in patients following alemtuzumab-mediated T-cell depletion in the context of allogeneic HSCT. The GPI-anchor negative T cells present in the blood of patients after allogeneic HSCT were responsible for higher cGVHD incidence. These gene variants could be used as predictors of development of cGVHD and inflammatory complications after allogeneic HSCT.

**0459**

**GENETIC VARIABILITY IN INNATE IMMUNE GENES (NLRP2, NLRP3, TGFBI) IMPACT THE ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) CLINICAL OUTCOME AND INFLUENCE THE INFLAMMATORY CYTOKINE PROFILE PRODUCTION**

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Background. Graft versus leukemia effect (GvL) is the main advantage in the allogeneic stem cell transplantation (allogeneic HSCT) setting. However in spite of this, the inflammatory response due to the conditioning regimen and the graft versus host disease (GvHD) are responsible for the transplant related mortality (TRM) incidence. Previous results by our group have shown variants of innate immune genes associated with GvHD incidence and higher cGVHD, causing high levels of inflammatory cytokines correlate with NLRP2, NLRP3, TGFBI gene expression. Results. We determined single nucleotide polymorphisms (SNPs) in genes involved in innate immune response. A total of 35 polymorphisms (32 SNPs and 3 insertions/deletions) at 15 candidate genes were analysed by high throughput mass array Sequenom TM platform or by DHPLC. Results. We found that a C to G rs2180314 SNP at Glutathione Transferase A2 (GSTA2) locus (Codon 112 which leads to a Ser to Thr aminoacidic transition) impacts OS and TRM in the whole population (CC vs G-carriers: HR=1.992, 95%CI=1.100-3.609, p=0.023 for TRM). Such an effect was particularly evident in patients who received busulfan (CC vs G-carriers: HR=2.438, 95%CI=1.446-4.108, p=0.0008 for OS and HR=4.005, 95%CI=2.005-10.461, p=0.0003 for TRM). No effect was present in the group not receiving busulfan. The polymorphism at microsomal GST-1 promoter (rs7790708) also affects OS and TRM, although to a lesser extent (AA vs G-carriers: HR=1.405, 95%CI=1.076-1.835, p=0.012 for TRM and HR=1.255, 95%CI=1.050-1.499, p=0.012 for OS). Summary/Conclusions. These data point out that genetic variability at loci controlling GSH balancing may affect allogeneic HSCT outcome. This study could be validated on patients populations belonging to controlled clinical trials with the aim to predict toxicity after allogeneic HSCT.

**0457**

**GENETIC VARIABILITY IN INNATE IMMUNE GENES (NLRP2, NLRP3, TGFBI) IMPACT THE ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) CLINICAL OUTCOME AND INFLUENCE THE INFLAMMATORY CYTOKINE PROFILE PRODUCTION**

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Background. Graft versus leukemia effect (GvL) is the main advantage in the allogeneic stem cell transplantation (allogeneic HSCT) setting. However in spite of this, the inflammatory response due to the conditioning regimen and the graft versus host disease (GvHD) are responsible for the transplant related mortality (TRM) incidence. Previous results by our group have shown variants of innate immune genes associated with GvHD incidence and higher cGVHD, causing high levels of inflammatory cytokines correlate with NLRP2, NLRP3, TGFBI gene expression. Results. We determined single nucleotide polymorphisms (SNPs) in genes involved in innate immune response. A total of 35 polymorphisms (32 SNPs and 3 insertions/deletions) at 15 candidate genes were analysed by high throughput mass array Sequenom TM platform or by DHPLC. Results. We found that a C to G rs2180314 SNP at Glutathione Transferase A2 (GSTA2) locus (Codon 112 which leads to a Ser to Thr aminoacidic transition) impacts OS and TRM in the whole population (CC vs G-carriers: HR=1.992, 95%CI=1.100-3.609, p=0.023 for TRM). Such an effect was particularly evident in patients who received busulfan (CC vs G-carriers: HR=2.438, 95%CI=1.446-4.108, p=0.0008 for OS and HR=4.005, 95%CI=2.005-10.461, p=0.0003 for TRM). No effect was present in the group not receiving busulfan. The polymorphism at microsomal GST-1 promoter (rs7790708) also affects OS and TRM, although to a lesser extent (AA vs G-carriers: HR=1.405, 95%CI=1.076-1.835, p=0.012 for TRM and HR=1.255, 95%CI=1.050-1.499, p=0.012 for OS). Summary/Conclusions. These data point out that genetic variability at loci controlling GSH balancing may affect allogeneic HSCT outcome. This study could be validated on patients populations belonging to controlled clinical trials with the aim to predict toxicity after allogeneic HSCT.
Histone deacetylase inhibitors (HDIs) are a new class of potential anticancer agents that are also active against leukemic cell lines and primary leukemia cells. The list of this class of drugs is growing and several are now in phase I/II clinical trials. Although the mechanism of action of HDIs on cancer cells may include enhancement of apoptosis, induction of cell cycle arrest and promotion of cellular differentiation, the exact mechanism is not known. Interestingly, researchers have found that in vivo expansion cultures addition of HDIs to the cultures delayed differentiation and enhanced stem cell numbers. Therefore, we investigated the effect of HDIs and other small molecules on normal hematopoietic stem cells in expansion cultures. To study this, we used a differentiation model where murine Lin-Sca-1+c-kit+ (LSK) cells are forced to differentiate in response to SCF and GM-CSF. It was found that Valproic Acid (VPA), together with LiCl, delayed hematopoietic stem cell (HSC) differentiation. Morphology and several in vitro and in vivo assays suggested that VPA and LiCl synergistically delayed or prevented differentiation and this was found also on the level of gene expression. Li did not significantly change gene expression during 7 days of culture, VPA affected expression of approximately 100 genes and the combination led to altered expression of more than 300 genes. The transcriptional program associated with multilineage differentiation has been affected. Moreover, the combination of VPA and Li enhanced the expression of multipotency-associated genes but reduced the expression of differentiation-associated genes. Our preliminary data also suggest that VPA delayed myeloid and lymphoid differentiation, whereas it enhanced erythroid differentiation. We further investigated whether other HDIs were able to modulate GM-CSF-induced differentiation. Therefore, we have chosen 6 different HDIs, VPA, sodium butyrate, trichostatin A (TSA), suberylanilide hydroxamic acid (SAHA), MS-275 and apicidin and compared their effects. All HDIs influenced cell growth, stem cell phenotype and delayed stem cell differentiation in a dose-dependent manner. Strikingly, expression of the mouse stem cell antigen (Sca)-1 could be upregulated by HDIs and this correlated with an increase in progenitor cell function. Progenitor cells were found to be an important target cell for HDI action because they increased their proliferative capacity in colony and single cell assays. This research implies that HDIs may be used to improve HSC expansion protocols by delaying differentiation and enhancing the potency of hematopoietic progenitors.

0462 THE PHENOTYPICALLY NAIVE T CELL SUBSET OF HEALTHY DONORS CAN BE USED TO EXPAND ACUTE MYELOID LEUKEMIA-REACTIVE CD4+ T CELLS IN VITRO

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Background. In allogeneic hematopoietic stem cell transplantation (allo-HSCT), donor-derived T lymphocytes recognize leukemia-associated minor histocompatibility antigens (mHag) on recipient leukemia cells, thereby mediating the graft-versus-leukemia (GvL) effect. Here, previous research has mainly focused on CD8+ cytotoxic T cells as GvL effectors. Other studies have demonstrated that CD4+ T cells are not only required to provide help for CD8 responses, but can exhibit direct cytolytic reactivity against leukemia cells. Furthermore, leukemia-reactive CD4+ T cells have been isolated from allo-HSCT patients upon GvL responses, and were successfully used to identify HLA class II-restricted mHags. Aim. We aimed at extending our recently developed protocol for CD8+ T cell expansion (Distler et al. Exp Hematol 2008; Albrecht et al., Cancer Immunol Immunother 2011) to the in vitro expansion and generation of acute myeloid leukemia (AML)-reactive CD4+ T cells from the naive donor T cell repertoire. Methods. Naive CD4+ T cells were isolated from PBMC of healthy donors either immunomagnetically (Naive CD4 T Cell Isolation Kit, Miltenyi Biotec) or by FACS sorting according to expression of CD45RA. T cells were stimulated in 96-well mini-mixed lymphocyte cultures ("mini"-MLLC) with HLA-DR and -DQ matched irradiated AML blasts isolated from patients at initial diagnosis. AML blasts were pre-cultured overnight in medium only, or for 4 days in medium supplemented with IL-4, GM-CSF, SCF and TNF-α to improve the antigen-presenting cell phenotype. After 2 weeks re-stimulations with AML blasts, mini-MLLCs were screened in split-well IFN-γ ELISPOT assays for reactivity against stimulator cells. Leukemia-reactive populations were expanded by further re-stimulations with AML blasts and were characterized for cytokine production, HLA restriction, cytolytic activity, and T cell receptor (TCR) β chain usage. Results. In 2 out of 3 AML patient/donor pairs with full HLA-DR and -DQ match several CD4+ T cell populations with strong and persistent AML reactivity could be expanded to cell populations exceeding >10⁸. Reactivity of these T cell clones was restricted by HLA-DR, -DQ or -DP alleles as determined by using specific HLA blocking antibodies in IFN-γ ELISPOT assays. Since HLA-DR was not yet typed, we cannot exclude allo-HLA-DR mismatch reactivity for HLA-DR-restricted T cell populations. T cells recognized either solely AML blasts, or also EBV-transformed B cells of patient origin in IFN-γ ELISPOT assays. Donor-derived EBV-B cells or K562 cells were not recognized. Chromium-release assays showed that CD4+ T cells lysed primary AML blasts only at moderate levels. Leukemia-reactive CD4+ T cell populations expressed either TCRs with β chain of a single family, indicating clonality, or were oligoclonal with up to 4 different TCR β chains. Summary/Conclusions. We show herein that leukemia-reactive CD4+ T cells can be readily isolated and expanded from naive precursors of HLA class II-matched healthy individuals by in vitro stimulations with primary AML blasts. Our current effort is to optimize the protocol in further patient/donor combinations. We also plan to investigate the in vivo behavior of AML-reactive CD4+ T cells in immunodeficient NOD/SCID-IL2Rγc (null) mice. Moreover, well-expanded leukemia-reactive CD4+ T cell clones can be used to identify potential target antigens for AML immunotherapy.

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COMBINATION CELL THERAPY OF EX-VIVO EXPANDED REGULATORY T CELLS AND HUMAN ADIPOSE TISSUE-DERIVED MSCS EFFECTIVELY INHIBITS ACUTE GRAFT-VERSUS-HOST DISEASE IN MURINE MODEL

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Background. Graft-versus-host disease (GVHD) remains the major barrier to the success of allogeneic hematopoietic stem cell transplantation (HSCT). The immunomodulatory effects of MSCs have been proposed as a promising treatment for acute GVHD as well as experimental encephalomyelitis and diabetes. However, the specific mechanisms involved in the immunoregulatory activity of MSCs remain unknown. Although the therapeutic effects of MSCs have varied in preclinical models those were closely related to the involvement of CD4+CD25+Foxp3+ regulatory T cells (Tregs) regardless of antigen specificity. Our previous study showed a negative effect of single infusion of MSCs on a murine model of collagen-induced arthritis (CIA) because MSCs alone did not prevent proinflammatory cytokines. Therefore, the combination cell therapy of ex-vivo expanded Tregs and MSCs may be expected synergistically to inhibit acute GVHD in allogeneic HSCT. Aims. The present study is aimed to evaluate the therapeutic efficacy of combination cell therapy of ex-vivo expanded regulatory T cells and human adipose tissue-derived mesenchymal stem cells (hAd-MSCs) to prevent acute GVHD in murine model. Methods. To obtain Treg cells, isolated CD4+ T cells from recipients (BALB/c) were cultured with anti-CD3 (1 ug/ml), anti-CD28 (1 ug/ml), human recombinant IL-2 (10 U/ml) for 3 days. In murine models of GVHD, lethally irradiated BALB/c mice are transplanted with CD4+CD25+ T cells from recipients (BALB/c) were cultured with anti-CD3 (1 ug/ml), anti-CD28 (1 ug/ml), human recombinant transforming growth factor (5 ng/ml) and all-trans retinal (1 U|M) for 3 days. In murine models of GVHD, lethally irradiated BALB/c mice were transplanted with CD4+CD25+ regulatory T cells on day 0. Following transplantation, recipients were transplanted with 4 times combinations of hAd-MSCs and Tregs or single hAd-MSCs injection. All animals were monitored for survival and clinical signs of GVHD. Results. The results showed that combination cell therapy of hAd-MSCs and Tregs can act remarkably protected recipients from lethal GVHD and prevented severe tissue damage after allogeneic BM transplantation. A single infusion of hAd-MSCs was less effective than those in preventing GVHD. These therapeutic effects were associated with an increase of Th2 and Tregs for suppressive effect against activated T cells, and decrease of Th1 and Th17 cells. Conclusions. These data indicate that combination cell therapy of ex-vivo expanded regulatory T cells and hAd-MSCs effectively inhibits acute graft-versus-host disease in murine model. In addition, the results of the ongoing clinical trials will properly assess the therapeutic potential of hAd-MSCs and Tregs.
THE INVESTIGATION OF GENOMIC PROFILES IN PLASMA CELL LEUKEMIAS BY MEANS OF AN INTEGRATIVE MICROARRAY APPROACH

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Background. Multiple myeloma (MM) is a clonal proliferation of malignant plasma cells (PCs) characterized by a marked genomic instability. Plasma cell leukemia (PCL) is an aggressive malignancy that can occur directly (primary, pPCL) or progress from intramedullary MM (secondary, sPCL). Genome-wide studies in PCL are still limited. Aims. To provide insights into the genomic lesions and altered molecular pathways characterizing Plasma Cell Leukemia. Methods. Highly purified PCs from 34 newly diagnosed pPCL and 16 untreated pPCL patients were characterized for the main chromosomal aberrations by FISH. pPCL cases were recruited in a multicenter GIMEMA clinical trial testing the lenalidomide/low dose dexamethasone combination. Gene expression profiles were generated on Gene 1.0 ST array (Affymetrix). Results. Unsupervised analysis of gene expression between pPCL and MM, MM and sPCL, and pPCL and sPCL, respectively, highlighted distinct expression profiles grouped most of PCLs and MMs into two distinct categories and partly (24%) associated to invasion and metastasis enriched in cytoskeleton organization, cell adhesion, migration categories. Supervised analysis evidenced 237 differentially expressed genes (DEGs) between the expression levels and the occurrence of allelic imbalances intriguingly, another showed a hyperdiploid pattern. A correlation between the expression levels and the occurrence of allelic imbalances was identified for 199 genes mainly localized in the previously described altered regions. The same integrative approach applied on miRNAs expression profiles was generated on miRNA Microarray V2 (Agilent). Genome-wide DNA profiles of 13 pPCLs were obtained using the 250K Nsp SNP array (Affymetrix); copy number values were inferred through circularly binary segmentation and FISH-based normalization. Genomic structural abnormalities in pPCL closely reflect expression imbalances. Specific gene and miRNA signatures, altered molecular pathways and novel genetic lesions potentially involved in more aggressive forms of PC dyscrasia.

0466 ROLE OF TORC1 AND TORC2 IN MULTIPLE MYELOMA

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Background. Mammalian target of rapamycin (mTOR) is a downstream serine/threonine kinase of the PI3K/Akt pathway that integrates signals from the tumor microenvironment such as cytokines and growth factors, nutrients and stresses to regulate multiple cellular processes, including translation, autophagy, metabolism, growth, proliferation, survival, migration, and invasion. Mutations in distinct multi-protein complexes, TORC1 (Raptor) and TORC2 (Rictor). Activation of TORC1 leads to the phosphorylation of p70S6 kinase and 4E-BP1, while activation of TORC2 regulates phosphorylation of Akt and other AGC kinases. In multiple myeloma (MM), PI3K/Akt plays an essential role enhancing cell growth and survival and is activated by the loss of the tumor suppressor gene PTEN and by the bone marrow microenvironment. Rapamycin analogues have been tested in clinical trials in MM and their efficacy as single agents is modest. Inhibition of Akt and 4E-BP1 signaling requires inactivation of both complexes TORC1 and TORC2. Consequently, there is a need for novel inhibitors that target mTOR in intact signaling complexes. Methods. MM cell lines and BM samples from MM patients. The mechanism of action was investigated by MIT, Annexin V, cell cycle analysis, Western-blotting and miRNA assays. For the in vivo analyses, OPM2 cells were injected into the tail vein of 30 SCID mice, the percentage of CD138+ cells and the effect in homing were detected by in vitro and in vivo flow cytometry, respectively. Nanofluidic proteomic immunoassays were performed in selected tumors. Results. We examined the protein expression levels of both mTOR complexes and the p-p70S6 kinase in the pPCL and sPCL. The IC50 of INK128 was in the range of 7.5-30 nM in the eight cell lines tested. Similar results were observed in freshly isolated plasma cells from MM patients. In the bone marrow microenvironment context, INK128 inhibited the proliferation of MM cells and decreased the p4E-BP1 induction. INK128 also showed a significantly greater effect inhibiting cell adhesion to BMSCs and HUVECs compared to rapamycin. These results are in concordance with our in vivo studies using in vivo flow cytometry showing that inhibition of both TORC1 and TORC2 had a significant effect in delaying homing of MM cells to the BM compared with the inhibition of only TORC1. Moreover, oral daily treatment with INK128 highly decreased the percentage of CD138+ tumor plasma cells in mice implanted with OPM2, reduced the levels of p-Akt and p-4E-BP1, and induced apoptosis upon BM homing studies. In conclusion, both TORC1/TORC2 and its downstream targets are major regulators of cell cycle, apoptosis and adhesion of MM cells. These results suggest that dual targeting of TORC1 and TORC2 by active-site mTOR inhibitors offers a novel therapeutic approach disrupting the interaction of MM cells with the BM microenvironment.

BKT140 IS A NOVEL CXCR4 ANTAGONIST WITH A POTENT STEM CELL MOBILIZATION CAPACITY AND THE ABILITY TO INDUCE MULTIPLE MYELOMA APOTOTIC CELL DEATH

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Background. CXCR4 is a member of the GPCR family that regulates the mobilization of hematopoietic stem cells (HSCs) in the bone marrow (BM) niche, as well as in the localization and adhesion of multiple myeloma (MM) tumor cells to the BM microenvironment. Therefore, blocking CXCR4 may result in mobilization of HSCs and may influence the biology of MM and the disease course. BKT140 is a high affinity CXCR4

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Aims. Evaluate the mobilization capacity and the ability to induce apoptosis of MM cells of BKT140, a novel CXCR4 antagonist. Methods. OPM2 and RPMI8226 cells were injected into the tail vein of 30 SCID mice. The percentage of CD138+ cells and the effect in homing were detected by in vitro and in vivo flow cytometry, respectively. Nanofluidic proteomic immunoassays were performed in selected tumors. Results. We examined the protein expression levels of both mTOR complexes and the p-p70S6 kinase in the pPCL and sPCL. The IC50 of INK128 was in the range of 7.5-30 nM in the eight cell lines tested. Similar results were observed in freshly isolated plasma cells from MM patients. In the bone marrow microenvironment context, INK128 inhibited the proliferation of MM cells and decreased the p4E-BP1 induction. INK128 also showed a significantly greater effect inhibiting cell adhesion to BMSCs and HUVECs compared to rapamycin. These results are in concordance with our in vivo studies using in vivo flow cytometry showing that inhibition of both TORC1 and TORC2 had a significant effect in delaying homing of MM cells to the BM compared with the inhibition of only TORC1. Moreover, oral daily treatment with INK128 highly decreased the percentage of CD138+ tumor plasma cells in mice implanted with OPM2, reduced the levels of p-Akt and p-4E-BP1, and induced apoptosis upon BM homing studies. In conclusion, both TORC1/TORC2 and its downstream targets are major regulators of cell cycle, apoptosis and adhesion of MM cells. These results suggest that dual targeting of TORC1 and TORC2 by active-site mTOR inhibitors offers a novel therapeutic approach disrupting the interaction of MM cells with the BM microenvironment.
inhibitor. The possible usage of BKT140 for mobilization of normal HSCs and as anti-MM compound was evaluated in patients with MM. Results. In pre-clinical study, we demonstrated that the CXCR4 antagonist BKT140 but not AMD3100 exhibited a CXCR4-dependent cytotoxicity toward MM cells. BKT140 induced apoptotic cell death of MM in vitro, demonstrating increased phosphatidylserine externalization, decreased intracellular membrane potential, caspase-3 activation, sub-G1 arrest, and DNA double-stranded breaks. In vivo, subcutaneous injections of BKT140 significantly reduced, in a dose-dependent manner, the growth of MM xenografts. Tumors from animals treated with BKT140 were smaller in size and weights, had larger necrotic areas and high apoptotic scores (p<0.01). Further, we conducted a phase I/IIa clinical study administering BKT140 to 18 MM patients (pts), assessing toxicity, mobilization of CD34+ cells, pharmacokinetic (PK), pharmacodynamic and its effect on CD138+ MM cells. BKT140 was administered at escalated doses (50,100,300,900 µg/kg) following high-dose cyclophosphamide (Cy) (2 g/m2) and G-CSF (5 µg/kg). G-CSF was started on day 5 post Cy and BKT140 was injected subcutaneously once on day 10. BKT140 demonstrated low toxicity and short PK profile. Preliminary results show that BKT140 administration resulted in a significant dose-dependent increase in PB CD34+ cells as well as neutrophils, monocytes and lymphocytes, compared to the Cy/G-CSF individual pt baseline. The mean absolute PB CD34+ cells mobilized following BKT140 administration was 6.6, 7.5, 11.2 and 20.5 x106/kg for the 4 BKT140 administered doses, respectively. Moreover, the number of aphaeresis was reduced from 2.5 to 1 procedure at the lowest (50 and 100 µg/kg) and highest (500,900 µg/kg) BKT140 doses, respectively. BKT140 at the highest doses reduced the number of PB CD138+ cells in PB in 3/7 pts with baseline CD138+ cells in their blood, while BKT140 at the lower doses increased the number of circulating CD138+ cells. The BKT140 mobilized grafts were used for AutoSCT in 15 MM pts following 200 mg/m2 melphalan conditioning. Pts received an average of 5.5x106 CD34+ cells/kg. All pts demonstrated rapid engraftment. The median day for neutrophil (>500/mm3) and platelet (>20,000/mm3, >50,000/mm3) recovery was day 11 (range, 0-13), day 11 (range, 0-14), and day 14 (range, 0-25), respectively. Conclusions. BKT140 demonstrated potent anti-MM effect in vitro and in vivo in mouse xenograft model. Furthermore, first human phase I/IIa clinical trial in MM pts showed that BKT140 can be safely administered with minimal toxicity and side effects. BKT140 significantly increased HSC mobilization and at higher doses reduced days of aphaeresis. In addition, at higher doses BKT140 released MM cells from the BM to the circulation. Additional studies are warranted to further evaluate the effect of BKT140 as an anti-MM agent.

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PROGNOSTIC MARKERS FOR THE PREDICTION OF EARLY RELAPSE IN MYELOMA PATIENTS ACHIEVING COMPLETE RESPONSE AFTER AUTOLOGOUS STEM-CELL TRANSPLANTATION

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Background. The incorporation of high-dose therapy/autologous-stem-cell-transplantation (HDT/ASCT) and novel agents for the treatment of young multiple myeloma (MM) patients have markedly improved response rates, particularly the achievement of complete response (CR), and survival. The association between higher CR rates and extended survival is unquestionable, but the clinical course of MM patients achieving CR is still heterogeneous; some patients die from disease progression within few months, whereas others live for over 10 years. Aims. To identify prognostic parameters for the prediction of early disease progression in patients achieving CR after HDT/ASCT. Methods. A total of 241 patients achieving CR at day+100 after HDT/ASCT are the focus of this study. Patients were included in two consecutive GEM/FETHEMA trials: GEM2000 (VBMCP/VBAD, n=140) and GEM2005<65y (Thalidomide/Dexamethasone, n=20; Bortezomib/Thalidomide/Dexamethasone, n=46; VBMCP/VBAD with Bortezomib in the two final cycles, n=35). All cases were referred for minimal residual disease (MRD) assessment by multiparameter flow cytometry (MFC) at day+100 after HDT/ASCT; baseline FISH analysis were available in 110 of the 241 patients. Results. Time-to-progression (TTP; median, 71 months) and overall survival (OS; 73% at 5-years) of these 241 patients were, as expected, superior than those of the whole series (TTP; median, 52 months; OS; 66% at 5-years). Multivariate analysis including those variables with significant influence in the univariate analysis showed that the best combination of independent predictive parameters for TTP were: MRD status by MFC (P=0.007; HR=9.004), FISH cytogenetics (high- vs. standard-risk; P=0.009, HR=9.081), and percentage of plasma cells in S-phase (>2%; P=0.013, HR=7.904); in turn, for OS MRD status by MFC (P=0.001; HR=7.730), FISH cytogenetics (P=0.011; HR=5.062) and age (<60 vs. ≥60 years; P=0.027, HR=5.420) were selected. We further investigated which parameters could help to identify those cases showing early progressive disease. Of the 241 patients, 30 (12%) progressed within one year after HDT/ASCT. This subgroup of patients showed significantly increased frequency of baseline anemia (48% vs. 26%, P=0.013), ISS stage 2 or 3 (66% vs. 55%, P=0.005), high-risk cytogenetics (40% vs. 10%, P=0.005) and persistent MRD detected by MFC (65% vs. 32%, P=0.001). By multivariate analysis, only MRD status by MFC (P=0.005, HR=4.686) and FISH cytogenetics (P=0.005, HR=4.528) were selected as independent prognostic factors for predicting progressive disease during 1-year after HDT/ASCT. Based on the variables with independent predictive value for early disease progression (MRD status by MFC and FISH cytogenetics), we established a predictive index by assigning 1 point for each adverse factor. Accordingly, 3 risk groups of patients in CR were defined, with significantly different (P<0.001) rates of disease progression within one year after HDT/ASCT for patients with no risk factors (4 progresses of 58 cases, 7%), cases with 1 risk factor (9 progresses of 45 cases, 20%), and patients with both risk factors (7 progresses of 7 cases, 100%). Conclusions. Patients with high-risk cytogenetics and persistent MRD after HDT/ASCT do not sustain the CR and are candidates for experimental consolidation treatments.
Acute myeloid leukemia - Clinical 1

0469
A VALIDATED DIAGNOSTIC MICROARRAY FOR NEWLY DIAGNOSED ADULT AML PATIENTS

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Background. In newly diagnosed acute myeloid leukemia (AML) gene expression (RNA) profiling (GEP) on Affymetrix GeneChips identifies homogeneous clusters that correlate with all favorable cytogenetic subtypes t(15;17), t(8;21) and inv(16)/t(16;16) and favourable CEBPA gene double mutants. GEP can also detect NPM1 type A/B/D by RNA genotyping with specifically designed probes on the microarray and it can assess the expression levels of individual transcripts with demonstrated prognostic significance (e.g. EVI1 and BAALC RNA expression). Aims. We set out to develop and validate an in vitro diagnostic microarray for use in diagnostics of newly diagnosed adult AML. Methods. A custom Affymetrix microarray, the AMLprofiler, was produced that contains a combination of generic and specially designed probes. The array was tested following hybridisation of 261 AML training cases. Next, the AMLprofiler was evaluated in an independent cohort of 267 unselected newly diagnosed cases of AML (Erasmus University Medical Center & University Ulm). Results. During validation in 267 independent cases the AMLprofiler identified 18/17 inv(16), 7/7 t(15;17) and 16/16 t(8;21) AML’s and 70/71 NPM1A/B/D cases. There was one false-positive inv(16) namely a t(11;16) translocation concurrent with MYH11 overexpression, suggesting involvement of the 16p13.1 breakpoint like in bona fide inv(16) or t(16;16) which has been infrequently reported in secondary AML. There was 1 false-negative case of NPM1 type-D, which prompted a retraining of the algorithm and subsequently required independent re-validation. This re-validation detected 68/66 NPM1 type A/B/D mutants in 143 Normal Karyotype AML cases. One of the two latter false-positive cases carried a non-AD type mutation which is clinically indistinguishable from D mutations. The EVI1 and BAALC cut points were validated according p < 0.05 in the logrank test for OS between high versus low expressing intermediate cytogenetic risk cases. Summary/Conclusions. We report the development of an AML gene expression RNA microarray for diagnostic use that can be applied by physicians in their own laboratories, to detect core binding AML, PML, NPM1 A/B/D mutant, CEBPA double mutant, high EVI1 and low BAALC AML cases for diagnostic use.

References

0470
CLOFARABINE + ARA-C IMPROVES RESPONSE RATES AND EVENT-FREE SURVIVAL, NOT OVERALL SURVIVAL, IN OLDER PATIENTS WITH RELAPSED/REFRACTORY AML COMPARED TO ARA-C ALONE: UPDATED CLASSIC III STUDY RESULTS

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Background. Prognosis among older patients (pts) with relapsed or refractory (R/R) acute myelogenous leukemia (AML) is dismal with median survival of 4.7 months (mo) (Rowe, Blood 2005 106: Abstract 546). For these patients, attainment of a complete remission (CR) is often considered the first treatment objective. Since remission status at the time of hematopoietic stem cell transplantation (HSCT), the only curative approach in R/R AML, is an important predictor of long term outcomes, new agents to increase complete remission rates prior to transplant are needed. Aim. Phase III study designed to evaluate the efficacy and safety of clofarabine (CLO) in combination with cytarabine (ara-C) compared to ara-C alone in older adult pts with R/R AML. Methods. This prospective, randomized, double-blind, placebo-controlled trial included pts ≥65 yrs with relapsed or refractory AML. Randomization to CLO-ara-C or placebo-ara-C was stratified by duration of remission following the first pre-study induction regimen [REF: refractory or CR1 <6 mos; REL: CR1 ≥6 mos]. Patients received CLO (40

Table 1.
mg/m² IV) or placebo followed by ara-C 1 g/m² IV daily x5 days. The primary endpoint was overall survival (OS). Select secondary endpoints included overall remission rate (ORR=CR+CRi), event-free survival (EFS) and safety; exploratory endpoints included H SCT rates. Results. Of the 320 pts with confirmed AML, 162 were randomized to CLO+ara-C and 158 to ara-C alone. The median age was 67 yrs (range: 55-86) and the median duration of their initial therapy and 49% had adverse cytogenetics. Although there was no difference in OS, CLO+ara-C demonstrated statistical significance across secondary efficacy endpoints, including doubling of remission rates and 37% improvement in EFS [HR: 0.63] (Table 1). Overall the number of patients who underwent H SCT was similar between the 2 arms; 21% in the CLO+ara-C arm vs 19% ara-C alone arms. However, a higher proportion of patients in the CLO+ara-C arm underwent H SCT while in remission from their study treatment (16% vs 9%). Overall 30-day mortality was 16% and 5% in the CLO+ara-C and ara-C-alone arms, respectively. Serious adverse events (SAE) occurred in 60% of the CLO+ara-C and 49% of the ara-C-alone pts. Serious infections occurred in 38% vs 22% of pts, respectively. The most frequent (≥5%) non-infectious SAE included febrile neutropenia (16% vs 12%) and pyrexia (4% vs 6%). Grade 3 or higher infections occurred in 65% vs 48% of pts, respectively. Grade 3 or higher non-infectious AEs occurring in ≥10% of pts in either arm included febrile neutropenia (47% vs 34%), hypothermia (26% vs 10%), hyperglycemia (14% vs 17%), anemia (13% vs 8%), neutropenia (11% vs 9%), increased AST (11% vs 2%) and increased ALT (10% vs 3%). Summary/Conclusions. While OS did not differ between arms, CLO+ara-C significantly improved response rates and EFS and allowed more patients to proceed to transplant in minimal residual disease study treatment compared to ara-c alone. Study follow-up continues and the role of clofarabine in the treatment of adult patients with AML continues to be investigated in randomized studies by cooperative groups.

0473
HIGH EXPRESSION OF THE BRCAL COMPLEX MEMBER BRE PREDICTS FAVORABLE PROGNOSIS IN ACUTE MYELOID LEUKEMIA, ESPECIALLY AMONG MLL-AF9 POSITIVE LEUKEMIA

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Background and Aims. Acute myeloid leukemia is a heterogeneous disease. Several recurrent genetic mutations that contribute to disease pathogenesis have an impact on disease outcome. Therefore, treatment strategies are influenced by the presence of mutations that have prognostic impact. Because deregulated expression levels of genes, like EVI1, also correlate significantly with disease outcome, the type of treatment is also influenced based on expression levels of individual genes. However, it is still not possible to classify all patients based on currently known aberrations. Changes in protein ubiquitination have recently been shown to contribute to the pathogenesis of acute myeloid leukemia (AML). To identify new prognostic factors in AML, we studied whether changes in expression of over 1600 ubiquitination-related genes correlated with clinical outcome in 525 adult AML patients. Methods and Results. Gene expression from 525 cases with AML, for whom written informed consent was obtained, was analyzed using Affymetrix HG-U133 plus 2.0 arrays. To study the correlation between expression changes of ubiquitination-related genes and overall survival (OS) the Cox proportional hazard model was used. In addition, we performed analyses to identify genes that showed altered expression in a small subset of patients (so called outlier expression) that correlated with survival. A differential expression of 9 genes was found to correlate with OS. Subsequent multivariate analyses identified the level of expression of five of these nine genes (BRE, DNM3T8, EVI1, KIFN68 and ZAP) as independent prognostic factors for overall survival. Outlier high expression of one of these genes, BRE, was observed in 8% of the patients and predicted a favorable overall and event-free survival (5-year overall survival of 57%). Importantly, high BRE expression was mutually exclusive with FLT3 ITD, CEBFA mutations, EVI1 over-expression, and favorable karyotypes. In contrast, high BRE expression co-occurred strongly with FAB M5 morphology and MLL-AF9 fusions. Strikingly, within the group of MLL-AF9 positive patients, high BRE expression predicted superior survival, while lack of high BRE expression predicted extremely poor survival (5-year overall survival of 80% vs 0%, respectively, p=0.0002). Finally, unsupervised gene expression profiling showed that 86% of the patients with high BRE expression were confined to a previously unrecognized cluster. Conclusion. We conclude that high BRE expression defines a novel good risk group among adult AML. This work contributes to further risk stratification and sub-classification of AML and may contribute to individualized treatment strategies.

0472
DNMT3A MUTATIONS IN ACUTE MYELOID LEUKEMIA: FREQUENCY AND PROGNOSTIC IMPACT

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Background. Acute myeloid leukemia (AML) is a heterogeneous neoplasm of the hematopoietic stem cell caused by mutations, deregulated gene expression and epigenetic modifications of genes leading to decreased differentiation of hematopoietic progenitor cells and increased proliferation. Recently, mutations in DNMT3A have been identified to occur in AML. Aims. The aim of this study was to analyze the frequency and prognostic significance of DNMT3A mutations in a large cohort of uniformly treated, well characterized AML patients. Methods. A total of 489 AML patients younger than 60 years were examined for DNMT3A mutations by direct sequencing. The prognostic impact of DNMT3A mutations was evaluated in the context of other clinical prognostic markers and genetic risk factors (cytogenetic risk group; mutations in NPM1, FLT3, CEBPA, IDH1, IDH2, MLL1, Nras, WT1, and WT1 SNPrs16754; expression levels of BAALC, ERG, EVI1, MLL5, NMI and WT1). Results. DNMT3A mutations were found in 87 out of 489 patients (17.8 %). The highest mutation frequency was found in cytogenetically normal (CN)- AML (71 of 261 patients, 27.2 %). Patients with DNMT3A mutations were found to be older, had higher WBC and platelet counts and more often had a normal karyotype. Additionally, patients with mutated DNMT3A were also more likely to have mutations in NPM1, FLT3, and IDH1 genes and had higher MLL expression levels when compared to patients with wildtype DNMT3A. Multivariate analysis demonstrated that DNMT3A mutations independently predicted a shorter overall survival (OS) (HR 1.59; 95% CI 1.15-2.21; P=0.005), but were not associated with relapse-free survival (RFS) or complete remission (CR) rate when the entire patient cohort was considered. In CN-AML patients, DNMT3A mutations independently predicted shorter OS (HR 2.46; 95% CI 1.58-3.83; P<0.001) and lower CR rate (OR 0.42; 95% CI 0.21-0.84; P=.015), but not RFS (P=.32). Additionally, within CN-AML patients, DNMT3A mutations had an unfavorable effect on OS, RFS, and CR rate in NPM1/FLT3ITD high risk but not in low risk patients. Conclusion. DNMT3A mutations are among the most frequent mutations in younger AML patients, and are associated with an unfavorable prognosis.

0473
NPM1 MONITORING ENABLES EARLY DETECTION OF IMPELLING RELAPSE IN ACUTE MYELOID LEUKEMIA FOLLOWING CONVENTIONAL CHEMOTHERAPY AND POST ALLOGENEIC STEM CELL TRANSPLANTATION

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Background. Relapse of disease remains the major reason for treatment failure in patients with acute myeloid leukemia (AML), even after allogeneic stem transplantation (SCT). Early detection of relapse using molecular techniques could enable early preemptive therapy, but so far, the number of suitable markers is limited. More recently, mutations of the nucleophosmin gene (NPM1) have been described. These mutations are amongst the most common changes in AML and have shown potential for minimal residual disease (MRD) detection. However, only few studies on NPM1-based MRD detection have been published. Aims. In this study we investigated the suitability of NPM1 as MRD-marker in a large cohort of AML-patients. Methods. 184 NPM1-
mutant AML patients (pts) (median age 53.5 yrs. (range, 21-81 yrs.)), treated in protocols of the Study Alliance Leukemia (SAL) were prospectively monitored. We developed an optimized assay for the sensitive cDNA based detection of the three most common NPM1-mutations using a Real-Time-Q-PCR with locked-nucleic acid (LNA) containing primer-probe designs. The threshold for molecular relapse (mol-Rel) was defined as a 10-fold increase of NPM1 transcript level compared to the lowest level achieved or levels with greater than 1%. Molecular non or partial responder were defined as cases without a significant decrease of NPM1 transcript levels or levels > 1% after completion of the first line therapy. Results. We studied 184 patients having one of the three most common NPM1-mutations, A (N=156), B (N=17) and D (N=11). A total of 1661 samples (978 BM; 683 PBL) were analyzed, the median number of samples per patient was 7 (range, 3-55), the median molecular follow-up was 453 days (99-1703 days). 65 patients (35.3%) had undergone SCT (12 auto SCT, 53 allo SCT), an FLT3-ITD mutation was present in 68 pts. (37%). According to our criteria, 18 pts were defined as molecular non-responders and none of them achieved durable CR. In 28 pts with hematological relapse (hem-rel) and sufficient molecular follow-up, the rise of MRD preceded the hem-rel by a median of 66 days (range, 0-313 days). Out of 121 pts without mol-rel only one patient relapsed (p < 0.001). In a subgroup of 117 patients with available NPM-data in remission generalized linear models were fitted to model the risk of relapse in a defined time span. In a second step ROC-analyses were performed to identify MRD-ranges with different relapse risks. Conclusions. In conclusion, our data indicate that NPM1 mutations can serve as markers for MRD monitoring allowing early detection of recurrent disease in a considerable proportion of AML patients. Increasing NPM1 transcript level could trigger preemptive intervention using DLI after allogeneic SCT or targeted therapy within prospective clinical trial.

Non-Hodgkin Lymphoma - From biology to therapy

0474

MICRONRNAS PLAY A PIVOTAL ROLE IN REGULATING WALDENSTROM'S MACROGLOBULINEMIA BIOLOGY

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Background. Waldenström’s Macroglobulinemia (WM) is a low-grade lymphoproliferative disorder characterized by the presence of a lymphoplasmacytic infiltrate in the bone marrow and serum monoclonal immunoglobulin M. Cytogenetic and molecular studies on gene expression analysis at the mRNA level have demonstrated minimal changes in WM cells. Therefore, multi-level characterization of this disease at genetic and epigenetic levels is required to improve our understanding of the underlying molecular changes that lead to the initiation and progression of this disease. We have therefore evaluated how microRNA (miRNA) aberrations may pose the modulation of WM biology both in vitro and in vivo. Aims. 1) To determine miRNA profiling in primary WM cells. 2) To evaluate the functional role of miRNA-155 and -9* in regulating WM biology both in vitro and in vivo. Methods. miRNA- and gene-expression-profilings have been performed on bone-marrow-derived-CD19+ WM cells, compared to their normal cellular counterparts. Data were validated by stem-loop-qRT-PCR. In vitro and in vivo functional studies were performed on precursor-anti-miRNA-155 and precursor-miRNA-9*-transfected-WM cells. Effect on signaling cascades have been evaluated by western-blot and immunofluorescence. DNA-proliferation, cytotoxicity, cell cycle, apoptosis were assessed by thymidine incorporation, MTT, PI, Apo2.7 staining, respectively. GFP+ WM cells were transfected using either control-probe or miRNA-155 knockdown probe, and then injected in mice 24 hours after transfection: in vivo confocal imaging has been performed. Results. WM cells present with a miRNA signature characterized by increased expression of miRNA-155 and decreased expression of miRNA-9* (ANOVA; P< 0.01). Potential microRNA-155 target genes were identified using gene-expression-profiling and included genes involved in cell cycle progression, adhesion, and migration. Predicted miRNA-9* included histone-deacetylases (HDAC4; HDAC5) and -acetyltransferases (Myst3). We found that miRNA-155 regulates proliferation and growth of WM cells in vitro and in vivo by inhibiting signaling cascades including MAPK/ERK, PI3/AKT and NF-kB pathways. In addition, we demonstrated that primary WM cells are characterized by unbalanced expression of HDACs and HATs at gene level, responsible for decreased acetylated-histone-H3 and -H4, at protein level and increased HDAC activity. miRNA-9* played a functional role in regulating histone-acetylation and HDAC activity in WM cells, based on their ability to target HDACs and HATs, leading to induction of toxicity in precursor-miRNA-9*-transfected cells, as shown by reduced proliferation rate, cell cycle arrest, induction of apoptosis, supported by PARP-, caspase-8-, caspase-9-clearage. In addition, miRNA-9* induced autophagy in WM cells by modulating Rab7 and LC3B. Conclusion. These in vitro and in vivo findings confirm that miRNA-155 and -9* are crucial regulators of WM pathogenesis; and provide the basis for miRNA-based-therapeutic strategies in this disease.

0475

DEPLETION OF TUMOUR ASSOCIATED MACROPHAGES SIGNIFICANTLY RETARDS THE PROGRESSION OF AN AGGRESSIVE AND CHEMORESISTANT MODEL OF B-CELL NON HODGKIN LYMPHOMA

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Background. A large number of infiltrating tumour associated macrophages (TAM) is associated with a poor prognosis in many cancers. In vitro studies have established the diversity and plasticity of macrophages, such that they may exhibit classically activated (M1) or alternatively activated (M2) phenotypes. In models of non-lymphoid malignancies, TAM exhibit pro-tumoural features approximating an M2 phenotype. In Non-Hodgkin Lymphomas (NHL) there are conflicting reports of the clinical significance of TAM
serum cytokine levels were measured by FACS analysis and ELISA, immunohistochemistry. Circulating monocyte populations and by FACS analysis of single-cell suspensions of lymph nodes, and by cellular composition of the immune microenvironment were assessed populations, were determined by real-time PCR. Changes to the cellular landscape to study B-NHL we used a mature B-cell lymphoma arising in Eμ-myc/bcl-2 transgenic mice, which, when intravenously injected into healthy C57BL/6 mice produced a disseminated lymphoma. Macrophage depletion was achieved by intravenous injection of liposomes containing dichloromethylene-diphosphonate (Liposomal Clodronate). A variety of schedules of delivering Liposomal Clodronate were employed to establish the nature and amplitude of effects on progression of lymphoma. Subsequent studies employed macrophage depletion with Liposomal Clodronate combined with adoptive transfer of syngeneic BM derived macrophages (BMDM), in vitro polarized to M1 and M2 phenotypes. More specific targeting of TAM, and relative sparing of physiological monocytes and resident tissue macrophages, was attempted by pharmacological inhibition of monocyte recruitment. This strategy was used against growing lymphomas, and in lymphomas relapsing after chemotherapy. Lymphoma growth was assessed by measuring lymph node weight, and cross-sectional area in tissue sections. Gene expression changes in whole lymph node tissues with and without lymphoma, and following interventions to manipulate macrophage populations, were determined by real-time PCR. Changes to the cellular composition of the immune microenvironment were assessed by FACs analysis of single-cell suspensions of lymph node tissues, and by immunohistochemistry. Circulating monocyte populations and serum cytokine levels were measured by FACs analysis and ELISA, respectively. Results. Intravenous delivery of Liposomal Clodronate in mice injected with Eμ-myc/bcl-2 lymphoma successfully depleted macrophages in the bone marrow, lymph node, and spleen, and significantly reduced lymphoma mass compared to vehicle controls (29.7%). We observed a dose-response reduction in lymphoma growth in Liposomal Clodronate treated animals. Adoptive transfer of M1-polarized BMDM also attenuated lymphoma growth (37.3%). Moreover, it resulted in further attenuation of lymphoma growth in mice previously treated with Liposomal Clodronate (47.1%). Pharmacological inhibition of macrophage recruitment resulted in reduced lymphoma growth in otherwise untreated lymphomas (19.7%), as well as in lymphomas relapsing following cytotoxic chemotherapy, whilst not depleting the circulating monocyte pool. Summary/Conclusions. Out in vivo studies support a crucial relationship between macrophage numbers/phenotype and lymphoma progression. Therefore, targeting TAM provides a very attractive therapeutic opportunity in human B lymphomas.
method to retain the binding activity of each constitutive Fab. Cognate CH3-AD2-IgG and CH1-DDD2-Fab modules were generated and combined under mild redox conditions to produce 74-(20)-(20), comprising four Fabs of veltuzumab linked to milatuzumab, and 20-(74)-(74), comprising four Fabs of milatuzumab linked to veltuzumab. The in vitro activities of 74-(20)-(20) and 20-(74)-(74) were assessed in three MCL lines (JeKo-1, Mino, and Granta-519) for growth inhibition by cell proliferation assay and for apoptosis by annexin binding assay and the results compared with the monospecific counterparts of 74-(74)-(74) and 20-(20)-(20). In addition, the effects on human B cells and growth of JeKo-1 cells in whole blood were analyzed ex vivo. Results. Each HexAb was shown to be homogeneous, with >95% purity by size-exclusion HPLC and SDS-PAGE. Both 20-(74)-(74) and 74-(20)-(20) potently inhibited the growth of JeKo-1, Mino and Granta-519 cells at 10 nM. In contrast, neither parental antibody, alone or in combination, nor the two monospecific counterparts, 74-(74)-(74) and 20-(20)-(20), inhibited the growth of JeKo-1 under the same conditions, suggesting the requirement of heteromerization of CD74 and CD20 for the observed cytotoxicity. The two anti-CD20/CD74 HexAbs also induced 25-30% apoptosis in Jeko-1, compared to 10-12% apoptosis with parental IgG, alone or in combination, and similar results were observed in clinical samples obtained from MCL patients. Additional studies revealed that the bispecific HexAbs, but not the parental mAbs, induced strong homotypic adhesion, pronounced phosphorylation of ERKs and JNKs, and reduction of the anti-apoptotic protein Bcl-xl in target cells. Although both bispecific HexAbs were capable of depleting human B cells ex vivo, only 20-(74)-(74) inhibited the growth of JeKo-1 cells in blood. We also found that 20-(74)-(74) had a higher antibody-dependent cellular cytotoxicity than 74-(20)-(20), but neither showed complement-dependent toxicity. The in vivo efficacy of 20-(74)-(74), given 370 µg twice a week for two weeks, was demonstrated in nude mice bearing JeKo-1 xenografts, resulting in 56% increase in median survival as compared to saline control mice (P<0.0001). Conclusions. The promising results obtained for 20-(74)-(74) against MCL lines and clinical samples warrant its further preclinical evaluation as potential therapeutic against MCL and B-cell lymphomas that are refractory or poorly responsive to anti-CD20 or anti-CD22 antibodies.

0478

GERMINAL CENTER B-CELL SIGNATURE IS ASSOCIATED WITH HIGHER [18F]-FDG UPTAKE AND IMPROVES THE PROGNOSIS VALUE OF TEP SCAN IN DLBCL TREATED BY RITUXIMAB AND ANTHRACYCLINES-BASED CHEMOTHERAPY

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Background. In addition to the molecular classification, [18F]-Fluorodeoxyglucose positron emission tomography (FDG-PET) imaging is essential to optimise initial staging or to predict prognosis of DLBCL. Aims. The aim of the study was to assess the relationship between cell of origin (COO) classification and PET scan features in DLBCL. Methods. Fifty seven cases treated by CHOP/CHOP-like+R were retrospectively analysed (median age = 65y, aIPI 0-1 = 30%, 2-3 =70%). PET scan results at diagnosis (SUVmax), following 3/4 cycles of chemotherapy (interim PET) and at the end of treatment (final PET) were correlated to molecular features. Expression profile of 18 genes related to GCB/ABC signatures and 5 genes coding for glucose transporters (GLUT) was determined from frozen tissues using an Illumina platform and DASL technology (cDNA-mediated Annealing, Selection, Ligation and extension). Phenotypes were also assessed by immunohistochemistry (IHC) according to Hans algorithm. Results. Gene expression profiling classified 30 DLBCL in the GCB subtype (2-year PFS=76%) and 27 in the ABC subtype (2-year PFS=51%, p=0.03), giving a concordance rate of 77% with IHC. Expression of GLUT2 was significantly higher in DLBCL with SUVmax ×3 quartile, regardless the GCB/ABC subtype. At base-line, SUVmax was higher in the GCB subtype as compared to the ABC subtype (p = 0.029) but was not predictive of the outcome. Interim and final FDG-PET (negative / positive) were highly predictive of the prognosis. Using semi-quantitative assessment of SUV decrease at interim PET (SUV fast (n=36) and slow (n=9) responders (SUV ≥ or < 70%) were defined. In multivariate analysis, GCB/ABC(OR=5.1), aIPI(OR=7.1) and slow/fast responses (OR=0.1) were independently correlated with PFS and OS. Using the GCB/ABC classification and interim PET, we identified patients with a very favourable outcome (2-year OS/PFS = 100%) characterized by a very favourable outcome (2-year OS/PFS = 100%) characterized by a
PROGNOSTIC IMPACT OF PARTIAL OR TOTAL MONOSOMY 7 AS A SINGLE ANOMALY IN PRIMARY MDS


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Introduction. Partial (del(7q)) or total monosomy 7 (-7) is one of the most frequent cytogenetic abnormalities in MDS, occurring as an isolated anomaly in about 5% of abnormal cases in patients (pts) with primary MDS. The IPSS defines any abnormality of chromosome 7 as an unfavourable and classifies them, combined with complex abnormalities, into the poor risk cytogenetic subgroup. However, in previous publications from other groups, the prognosis of isolated -7/del(7q) was described as intermediate. The aim of the present study was to re-analyze the prognostic impact of -7/del(7q) as a single anomaly based on a large, international MDS database. Materials and Methods. 2901 Patients derived from the international MDS database were screened for monosomy 7. This large international data collection contains patients with MDS, originating from the German-Austrian (GA), - the International MDS Risk Analysis Workshop (IMRAW), the Spanish Cytogenetic Working group (GCECGH) and the International Cytogenetics Working Group of the MDS Foundation (ICWG). Only patients with primary MDS, age >=16, and bone marrow blasts <=30%, treated with supportive care exclusively, were considered for the analysis.Uni- and multivariate analyses were performed for overall survival (OS) and risk of AML-transformation. In multivariate analysis, site, age, gender, bone marrow blast count, date of first diagnosis and number of peripheral cytopenias were defined as co-variables. Results. In total, 59 patients (2.1% of all pts/4.6% of abnormal cases) with monosomy 7 were identified (del(7q), n=15; -7, n=44). The median age of these patients was 66.1 years, which is significantly lower compared to patients without monosomy 7 (70.0 years; p<0.01). Regarding peripheral blood count, the mean hemoglobin in -7/del(7q) pts (9.2 g/dl) as well as ANC (1.7*10^3/ul) did not differ significantly as compared to pts without -7/del(7q) whereas the platelet count in pts with -7/del(7q) was significantly lower (82*10^3/ul vs. 125*10^3/ul; p<0.01). The median overall survival in -7/del(7q) pts was 16.0 (95% CI 14.0-21.4) months and the Hazard ratio (HR, as compared to a normal karyotype with a median survival of 47.4 [44.0-53.4] months as the reference category) was 1.6 (1.1-2.5; p<0.01). According to the risk of AML-transformation, the median time to AML was 42.2 (14.4-not reached) months and the HR 1.7 (0.9-5.2; p=0.01). In comparison, this differed significantly from the median survival- (p<0.0001) and time to AML-transformation (p=0.027) for complex abnormalities, which are included with -7/del(7q) in the poor risk IPSS cytogenetic subgroup and were 6.7 (4.3-8.3) and 26.4 (14.0) months, respectively. The HR for complex abnormalities was 4.3 (3.4-5.4; p<0.01) for OS and 5.2 (3.8-7.5; p<0.01) for AML-transformation. Conclusions. The re-analysis of -7/del(7q), based on the largest MDS patient cohort yet published, confirms that the prognostic impact of an isolated total or partial monosomy 7 for overall survival as well as the risk of AML-transformation is not as poor as defined in the IPSS and significantly different from complex abnormalities. This finding is anticipated to be considered in the upcoming revision of the IPSS.

Acknowledgements: The authors like to thank the MDS-Foundation for its support.
Background. Most studies evaluating prognosis in MDS have focused on untreated patients from all subgroups. Higher transfusion burden and bone marrow blast percentage, and complex cytogenetics at baseline have been associated with reduced OS and increased AML progression in MDS patients (Greenberg P, et al. Blood. 1997;90:2079-86; Malcovati L, et al. J Clin Oncol. 2007;25:3503-10). Evaluation of predictive factors specific to disease-modifying drugs such as LEN, and to MDS subtypes such as Low-/Int-1-risk groups with del(5q) is needed. Aim. To identify predictive factors for OS and AML progression in RBC-transfusion dependent patients with IPSS-defined Low-/Int-1-risk MDS with del(5q), treated with LEN in 2 multicenter trials^MDS-003 (phase 2 single-arm) and MDS-004 (phase 3 randomized, double-blind). Methods. Patients who provided informed consent received LEN 5 mg on days 1-28, or 10 mg either on days 1-21 or 1-28 of 28-day cycles. Cox proportional hazards models assessed the impact of baseline characteristics and RBC-transfusion independence (TI) for ≥26 weeks, on OS and time to AML progression (calculated from study entry/randomization); RBC-TI for ≥26 weeks was included as a time-dependent covariate. Once potentially significant risk factors were identified, a multivariate model simultaneously determined the most important prognostic variables using a backward elimination variable-selection approach. The Table shows the univariate model with individual variables and the final model based on backward model selection. Results. From the 2 studies, 209 LEN-treated patients were included in the intent-to-treat population. Median age was 69 years (range 36-95); 70% of patients were female; 70% had isolated del(5q) and 26% had del(5q) plus ≥1 additional abnormality; 31%, 42%, and 6% had IPSS-defined Low-, Int-1-, and Int-2-/High-risk MDS, respectively. FAB subtypes were: 63% RA/RARS; 19% RAEB/CMML; and 19% other/missing. At baseline, median transfusion burden was 6 units/8 weeks (range 1-25) and median platelet count was 235×10^12/L (range, 14-1401). Median follow-up duration for OS was 38.4 months (range, 0.3-81.9) in MDS-003 and 36.1 months (range, 0.4-59.4) in MDS-004. Results of the Cox proportional hazards model are in the Table. Achieving RBC-TI for ≥26 weeks and higher baseline platelet counts were associated with significantly reduced relative risks of death (64% and 13% reductions per platelet count increase of 100×10^12/L, respectively). Additionally, significantly increases in the relative risk of death were reported with RAEB/CMML (63% increase), higher transfusion burden (6% increase per 1 unit/8 weeks), and older age (5% increase per year) at baseline. Higher transfusion burden and del(5q) plus ≥1 additional abnormality were associated with significantly increased relative risks of AML progression. Summary. In LEN-treated patients with Low-/Int-1-risk MDS with del(5q), higher baseline transfusion burden was associated with an increased relative risk of AML progression and death. Older age and RAEB/CMML at baseline were associated with reduced OS, whereas del(5q) plus ≥1 additional abnormality was associated with an increased risk of AML progression. Achievement of RBC-TI for ≥26 weeks and higher baseline platelet counts were associated with significantly increased OS in this large patient cohort, confirming the findings in previous, smaller-scale studies.

Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>0.99</td>
<td>0.97-1.01</td>
</tr>
<tr>
<td>Sex (male vs female)</td>
<td>1.02</td>
<td>0.98-1.06</td>
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<tr>
<td>IPSS risk (Low vs High)</td>
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<td>0.29-0.98</td>
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<tr>
<td>FAB classification (RAEB vs RARS)</td>
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<td>0.78-2.30</td>
</tr>
<tr>
<td>BAALC transcript levels (Low vs High)</td>
<td>0.16</td>
<td>0.05-0.46</td>
</tr>
<tr>
<td>EVI1 transcript levels (Low vs High)</td>
<td>0.09</td>
<td>0.02-0.40</td>
</tr>
</tbody>
</table>

A high 4-gene expression score is an unfavorable prognostic marker in MDS and is associated with a high risk for progression to AML. This prognostic marker may become useful for risk and treatment stratification.

**0482**

**A GENE EXPRESSION BASED RISK SCORE IN MDS PATIENTS PREDICTS AML TRANSFORMATION**


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**Background.** High expression levels of MN1, ERG, BAALC and EVI1 have been found to be associated with an adverse outcome in patients with acute myeloid leukemia (AML). Whether expression levels of these genes also have a prognostic influence in myelodysplastic syndromes (MDS) remains unknown. **Aims.** The aim of this study was to develop a scoring system that is predictive for progression of MDS to AML based on gene expression levels of MN1, ERG, BAALC and EVI1 in patients with MDS. **Patients and Methods.** MN1, ERG, BAALC and EVI1 transcript levels were analyzed by quantitative RT-PCR in 140 MDS patients and assessed for their prognostic significance in the context of other clinical and molecular markers. **Expression data of the four genes were combined in an additive score, which was validated in an independent patient cohort of 110 MDS patients. Results.** Patients with high compared to low expression of the individual genes MN1, ERG, BAALC or EVI1 showed a significantly worse overall survival (OS, P=.001; P<.001, and P=.044, respectively) and shorter time to AML progression (MN1, ERG, and BAALC, P<.001, EVI1, P=.001). Multivariate analysis revealed that a high 4-gene expression score, defined as expression above the median of at least two of the four genes predicted a significantly shorter OS (HR 2.29, 95%CI 1.29-4.08, P=.005) and time to AML progression (HR 4.83, 95%CI 2.01-11.57, P<.001) compared to a low 4-gene expression score, independent of karyotype, transfusion dependence, percentage of bone marrow blasts, ASXL1, and IDH mutation status. In a validation cohort of 110 MDS patients, a high 4-gene expression score predicted shorter OS (HR 1.77, 95%CI 1.04-3.04, P=.034) and time to AML progression (HR 3.6, 95%CI 1.17-7.65, P=.022). **Conclusion.** A high 4-gene expression score is an unfavorable prognostic marker in MDS and is associated with a high risk for progression to AML. This prognostic marker may become useful for risk and treatment stratification.
Background. ON 01910.Na is a new potent and selective mitotic inhibitor which inhibits the cell cycle progression at the G2/M interface, as well as the alpha and beta subunits of PI3-Kinase resulting in reduced levels of Cyclin D, C-myc and other regulatory proteins. It is also selective in cancer cells vs. normal cells. Methods. We analyzed bone marrow (BM) response and overall survival (OS) in 31 patients (pts) with refractory anemia with excess blasts (RAEB) -1,- 2 or -t, previously treated with azacitidine or decitabine who signed informed consent and were enrolled in 4 independent clinical trials. Pts received ON 01910.Na administered as a continuous intravenous infusion (CIV) from 2 to 6 days weekly or every other week (wk) with BM response initially assessed per protocol by wk 4 or 8 and every 8 wks thereafter.

Results. Median OS was 36 wks and reached 49 wks in patients treated with ON 01910.Na 1800 mg dosing per 24h over 3-day infusions every other wk. A BM complete response (CR) (>50% decrease from baseline BM blast and decrease below 5% for at least 4 wks, per MDS IWG 2006 criteria) or a > 50% decrease of BM blasts was documented in 13/24 (54%) treated pts with at least one follow-up BM evaluation (green line) and was associated with a 44-wk median overall survival (OS) by the method of Kaplan-Meier (Fig. 1). Patients with stable disease (N=9; orange line) had a 40-wk median OS. Two patients progressed (median OS=16 wks; brown line) and 7 were not assessed (median OS=7 wks; blue line). Five pts had complete BM response and 5 patients had a hematological improvement (IWG 2006). Best results were found with 3-day infusions. Overall, ON 01910.Na infusions were well tolerated and no myelotoxicity was found when analyzing bone marrow cellularity.

Conclusion. The median survival of MDS pts who failed to respond to prior treatment with azacitidine or decitabine has been reported to be approximately 4 to 6 months. These results and the apparent predictive value of BM response to ON 01910.Na for estimating overall survival of these patients have led to the initiation of a randomized survival trial of ON 01910.Na 3-day infusions vs. best supportive care in RAEB 1,2,t pts who failed or progressed after receiving hypomethylating agents.

Figure 1.
for nilotinib at both doses (vs imatinib) regardless of Sokal risk score. Rates of CCyR by 24 months were also significantly higher on both nilotinib arms (87%, 85%) vs imatinib (77%; $P = 0.0018$ and $0.0160$ for comparison with nilotinib 300 mg BID and 400 mg BID, respectively). Rates of CMR4 and CMR4.5 at any time were significantly higher for both doses of nilotinib vs imatinib (CMR4: 44%, 36%, and 20% for nilotinib 300 mg and 400 mg BID and imatinib, respectively, $P < 0.001$ for both), CMR4.5 (21%, and 10% and 12%, respectively, $P < 0.001$ and 0.0001 for nilotinib 300 mg BID and 400 mg BID vs imatinib, respectively). Progressions to AP/BC (without clonal evolution) continued to be less frequent on nilotinib, occurring in 2, 3, and 12 patients on the nilotinib 300 mg BID, 400 mg BID, and imatinib arms, respectively. At 24 months, OS remained higher in both nilotinib groups, but there were fewer CML-related deaths on both nilotinib 500 (n = 5) and 400 mg BID (n = 3) vs imatinib (n = 10). Twice as many patients had emergent BCR-ABL mutations on imatinib (n = 20) vs nilotinib (n = 10 and 8 for 300 mg BID and 400 mg BID, respectively) and mutations were more frequent in patients with high or intermediate Sokal risk. Overall, 72 (26%), 61 (22%), and 92 (35%) of patients in the nilotinib 300 mg BID and 400 mg BID and imatinib arms, respectively, discontinued treatment. Both drugs were well tolerated, with the fewest discontinuations due to adverse events/laboratory abnormalities in the nilotinib 300 mg BID arm (9% vs 15% and 10% for the nilotinib 400 mg BID and imatinib arms, respectively). There was less variation in the safety profile of nilotinib in the second year of treatment. Conclusions. With a minimum follow up of 24 months, nilotinib continues to demonstrate superior molecular responses and improved disease control compared with imatinib. These data continue to support nilotinib as a potential new standard of care in newly-diagnosed patients with Ph+ CML-CP.

**0485**

**THE BELA TRIAL: BOSUTINIB VERSUS IMATINIB IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA; 18-MONTH FOLLOW-UP**

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Background. Bosutinib is an orally active, dual Src/Ab1 tyrosine kinase inhibitor with minimal inhibitory activity against PDGFα or c-kit. Aim. The phase 3 BELA study compared bosutinib with imatinib in patients with newly diagnosed Philadelphia chromosome positive (Ph+) chronic phase chronic myeloid leukemia (CP CML). Methods. Patients (N = 502) randomized to oral bosutinib 500 mg/day (n = 250) or imatinib 400 mg/day (N = 252). Study design and endpoints have been described. Results. Of 1275 CP-CML patients analyzed (CML, n=1275; Ph+ ALL, n=164), 1439 patients were analyzed (CML, n=1275; Ph+ ALL, n=164). Safety analyses included all treated patients; efficacy analyses included all randomized patients (intent-to-treat [ITT] population). Results. Median treatment duration was 16.6 months for bosutinib and 16.8 months for imatinib; 69% and 78% of patients, respectively, were still receiving therapy. Common treatment-emergent adverse events (TEAEs; ≥20% of patients) observed with bosutinib and imatinib, respectively, were diarrhea (68%, 22%), vomiting (31%, 14%), nausea (31%, 35%), rash (21%, 16%), and muscle cramps (4%, 20%). Pleural effusions were seen in 5% of bosutinib patients (no imatinib patients). Grade ≥3 TEAEs (≥2% of patients) seen with bosutinib were diabetes (31%), edema (6%), and hypertension (5%). Twenty-two percent of bosutinib patients and 6% of imatinib patients discontinued due to AEs. Deaths occurred in 4 (1.6%) bosutinib patients and 12 (4.8%) imatinib patients; overall, 81% of these died from disease progression, with CML-unrelated deaths reported for only 1 bosutinib patient and 4 imatinib patients. Conclusions. The 3-year overall response (CCyR) rates for bosutinib and imatinib, respectively, at 1 year were 70% and 68% for the ITT population, and 78% and 68% for the evaluable population (P = 0.026). Cumulative CCyR rates by 1 year were 79% (bosutinib) and 75% (imatinib). Major molecular response (MMR) rates at 1 year were higher for bosutinib versus imatinib (93% vs 90% for bosutinib and imatinib, respectively, P = 0.053). Treatment failures were reduced in the bosutinib group compared with the imatinib group (3% vs 10%; P < 0.001). Event-free survival rates were similar between groups (92% for bosutinib and 90% for imatinib). Summary/Conclusions. Safety and efficacy were consistent with previously reported results. Bosutinib had a distinct and acceptable toxicity profile. Bosutinib showed a significantly higher MMR rate at 1 year, significantly faster times to CCyR and MMR, and a borderline significantly lower transformation rate versus imatinib. Conclusion, bosutinib may provide a new therapeutic option in patients with newly diagnosed Ph+ CP CML. Data for the 18-month follow-up will be presented.

**BCR-ABL KINASE DOMAIN MUTATIONS IN IMATINIB AND IN SECOND-GENERATION TYROSINE KINASE INHIBITOR ERAS: A REVIEW OF SEVEN YEARS OF MUTATION ANALYSIS BY THE GIEMSA CML WORKING PARTY**

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Background. Over the years, Bcr-Abl kinase domain (KD) mutation analysis has more and more extensively been applied in Ph+ patients receiving tyrosine kinase inhibitors (TKIs). Aim. We reviewed the database recording the mutation analyses performed in our laboratory from January 2004 to January 2011 in order to: i) assess how the clinical relevance of mutations (overall and individually) has changed from the imatinib to the 2nd-generation TKI era; ii) understand how the role of mutation analysis in routine clinical management of patients has evolved over time; iii) elucidate how frequently physicians may expect to find mutations in specific settings. Methods. 5285 bcr-Abl KD mutation analyses were performed by D-HPLC and/or direct sequencing; 1439 patients were analyzed (CML, n=1375; Ph+ ALL, n=164). Results: Since 2006, mutation analysis of CP-CML patients on imatinib has usually been triggered by failure or suboptimal response according to ELN definitions. Only 41/142 (29%) failures and 19/222 (10%) suboptimal responses we analyzed harbored mutations; the likelihood of mutation detection varied across different subcategories. In particular, no mutations were detected in any of the 42 CCyR patients who failed to achieve MMR at 18 months. Over time, more and more physicians asked for mutation analysis of their CP-CML patients because of a Bcr-Abl transcript increase at a single RO-PCR test; mutations were detected in 0/26 patients who experienced <1-log increase without MMIR loss, 1/41 patients who experienced ≥1-log increase with loss of MMR and 2/41 (5%) patients who experienced ≥1-log increase with loss of MMR but not CCyR. The 25 additional patients analyzed after a transcript increase confirmed by two subsequent RO-PCR assess-
ments were negative as well. Among imatinib-resistant CML patients receiving a 2nd-generation TKI (dasatinib, nilotinib), 38 analyses were triggered by provisional ELN criteria for failure or suboptimal response; again, failures were more frequently associated with mutations (57%) than suboptimal responses (21%). In addition, 71% of patients who lost a previously achieved response (HR or CyR) were positive for mutations. The most frequent mutations conferring resistance to dasatinib/nilotinib in CML and Ph+ ALL included T315I (30.3%), F317L (16.2%), Y253H (16.2%), F359V (7.1%), V299L (7.1%), E255K (6.1%), E255V (5.1%), F359I (4%), T315A (5%), F359C (2%) - detected either alone (56% of patients), or combined (29%), or together with other mutations (15%). Some physicians ask for mutations analysis of their newly treated, TKI-naive patients - mutations were detected in 1/58 CP CML, 3/12 BC CML, and 3/60 Ph+ ALL patients. Summary. Bcr-Abl KD mutations contribute differently to different types of ‘resistance’, and this happens both in the setting of imatinib first-line and in the setting of 2nd-generation TKIs second-line - although the mutation frequency is, overall, higher in the latter. In CP CML patients on imatinib who show Bcr-Abl transcript increase, only loss of MMR is a reasonable trigger for mutation analysis - although <3% of molecular suboptimal responders can be expected to harbour mutations. Prevalence of individual mutations is changing. Additional analyses will be presented.

Acknowledgments: Supported by ELN, AIL, PRIN.

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BCR-ABL FUSION TRANSCRIPT AND OUTCOME OF CHRONIC MYELOID LEUKAEMIA PATIENTS IN EARLY CHRONIC PHASE TREATED WITH IMATINIB: A GIMEMA CML WP ANALYSIS

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R Di Lorenzo,16 M Annunziata,11 E Usala,16 S Sica,13 P Galieni,14 E. Abruzzese,5 L Levato,6 F Cavazzini,7 E Orlandi,8 F Stagno,9
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With Imatinib: A GIMEMA CML WP Analysis

BCR-ABL fusion transcript and outcome of chronic myeloid leukemia patients in early chronic phase treated with imatinib: a GIMEMA CML WP analysis

The ten most frequent mutations conferring resistance to dasatinib/nilotinib in CML and Ph+ ALL included T315I (30.3%), F317L (16.2%), Y253H (16.2%), F359V (7.1%), V299L (7.1%), E255K (6.1%), E255V (5.1%), F359I (4%), T315A (5%), F359C (2%) - detected either alone (56% of patients), or combined (29%), or together with other mutations (15%). Some physicians ask for mutations analysis of their newly treated, TKI-naive patients - mutations were detected in 1/58 CP CML, 3/12 BC CML, and 3/60 Ph+ ALL patients. Summary. Bcr-Abl KD mutations contribute differently to different types of ‘resistance’, and this happens both in the setting of imatinib first-line and in the setting of 2nd-generation TKIs second-line - although the mutation frequency is, overall, higher in the latter. In CP CML patients on imatinib who show Bcr-Abl transcript increase, only loss of MMR is a reasonable trigger for mutation analysis - although <3% of molecular suboptimal responders can be expected to harbour mutations. Prevalence of individual mutations is changing. Additional analyses will be presented.

Acknowledgments: Supported by ELN, AIL, PRIN.

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CANCEROUS INHIBITOR OF PP2A (CIP2A) AT DIAGNOSIS OF CHRONIC MYELOID LEUKAEMIA IS A CRITICAL DETERMINANT OF DISEASE PROGRESSION

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Background. Chronic myeloid leukemia (CML) is characterized by the BCR-ABL fusion gene. Different types of BCR-ABL transcripts can be found, due to different genomic breakpoints and alternative splicing. The most frequent transcripts are the e1a2 (b2a2) and the e1a2 (b2a2). Occasionally, both transcripts may be present. In the imatinib (IM) era, few data about the prognostic significance of the transcript type are available, particularly in the setting of early chronic phase (ECP): one study suggested that patients with the b2a2 transcript may be more sensitive to IM (de Lemos et al. Genet Mol Res 2005), while two larger studies suggested that patients with b2a2 transcript may have better responses to IM (Vega-Ruiz et al. ASH 2007; Lucas et al. Haematologica 2009). No systematic evaluations in large prospective clinical trials have been performed. Aims. To investigate the influence of the BCR-ABL transcript type on the responses and the outcome of ECP CML treated with IM. Methods. Analysis of 3 concurrent clinical trials of the GIMEMA CML WP (Clin Trials Gov. NCT00514488, NCT00510926 and observational trial CML023). Response monitoring: conventional cytogenetic examination (bone marrow) and Q-PCR (peripheral blood). Definitions: Major Molecular Response (MMR): BCR-ABL/ABL ratio <0.1% (International Scale); failure: - - revisited IM and/or IM + interferon or + dasatinib (phase I/II); - - treatment discontinuation for any reason. All the calculations have been made according to the intention-to-treat principle. Results. 559 consecutive ECP CML patients were enrolled. Patients expressing rare transcript types (e1a2 and e19a2) and patients with both b2a2 and b3a2 transcripts were excluded: 495 out of 559 patients were evaluable, 205 (41%) with b2a2 transcript and 290 with b3a2 transcript (59%). The 2 groups were comparable (no significant differences in sex, age, Sokal/Hasford score distribution, clonal chromosomal abnormalities in Ph+ cells), except for the proportion of patients treated with IM 800 mg/daily: 20% and 28% (p=0.054) in patients with b2a2 and b3a2, respectively. The median observation time was 60 months. In patients with b2a2 and b3a2 transcript, the observed 12-months CCgR rates were 75% and 79%, respectively, with a cumulative CCgR incidence of 89% and 88%, respectively (no significant differences). The time to MMR was significantly shorter for patients with b3a2 transcript and the overall estimated probability of MMR was significantly lower for patients with b2a2 transcript (85% vs 90%, p<0.001, fig. 1). The probability of Overall Survival (OS), Progression-Free Survival (PFS), Failure-Free Survival (FFS) and Event-Free Survival (EFS) was 86% and 91% (p=0.064), 82% and 90% (p=0.027), 70% and 76% (p=0.095), 50% and 70% (p=0.027) in patients with b2a2 and b3a2 transcript, respectively (fig. 1). Summary/Conclusions. In patients with b2a2 and b3a2 transcript the CCgR rates were comparable, but the overall estimated probability of MMR was significantly lower for patients with b2a2 transcript. OS, FFS, FFS, and FES were uniformly lower in patients with b2a2 transcript (PFS and EFS: p<0.05). The b2a2 transcript is a candidate adverse prognostic factor in ECP CML patients treated with IM frontline.

Acknowledgements: European LeukemiaNet, COFIN, University of Bologna, BolognaAIL.

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Figure 1. Response and outcome.
achieve a CCR. With SET, protein levels were significantly higher at diagnosis in the CCR and No-CCR groups compared to normal values (p=0.001 and p=0.01 respectively), but in patients who subsequently progressed to BC there was a trend for lower SET levels - suggesting that SET was not solely responsible for inhibiting PP2A in destined to progress into BC. At diagnosis of CML, patients who later progress to BC have significantly higher levels of CIP2A protein (p<0.0001) than patients who do not progress. There was no correlation between CIP2A levels and patients’ Sokal score. CIP2A protein was not suppressed by imatinib treatment in vivo. During disease progression, CIP2A protein levels increased further, suggesting that PP2A function is increasingly impaired in these patients. Chronic phase patients who have high CIP2A protein at diagnosis have a 100% probability of progressing to BC - with the mean time to progression being 13 months. Knockdown of CIP2A resulted in increased PP2A activity and decreased BCR-ABL1 tyrosine kinase activity. mRNA expression of CIP2A, SET and PP2A had no prognostic value. These data show that two mechanisms control PP2A activity in CML. In patients who clinically respond well SET appears to be the controlling inhibitor, while in patients who progress to BC PP2A activity appears to be impaired predominantly by CIP2A. Importantly these two signalling mechanisms can be detected at diagnosis. Summary/Conclusion. CIP2A is a prospective biomarker of BC in CML and may be a useful therapeutic target.

Acute lymphoblastic leukemia - Biology

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Deregulated expression of microRNA125B by IGH transcription elements causes acute lymphoblastic leukemia in vivo
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Background and Aims. Chromosomal translocations involving the immunoglobulin heavy chain gene (IGH) locus play a pivotal role in the pathogenesis of human B-cell malignancies. IGH translocation brings target genes positioned on different chromosome loci into close apposition with transcription elements within the IGH locus, resulting in deregulated expression of the target genes. We previously reported an insertion of microRNA125b1 (hereafter, miR125b1) into the IGH locus in a patient with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Other groups identified fusion sequences of IGH and miR125b1 from t(11;14)(q24;q32) indicating that chromosome translocation involving IGH and miR125b1 loci is a recurrent event in human BCP-ALL. However, in vivo oncogenesis of miR125b in B-cells have not been fully understood. To confirm that deregulated expression of miR125b by IGH regulatory elements induces B-cell tumor, we generated transgenic mouse (Emu/miR125b1 TG mouse) mimicking the t(11;14)(q24;q32).

Methods. The transgene consisted of human intronic enhancer of IGH

Figure 1.
tumor progression. With distinct functional properties may preferentially contribute to preclinical models to design new therapeutic strategies. Genomic patients with acute lymphoblastic leukemia (ALL) still relapse and ex-vivo

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CLONAL SELECTION IN XENOGRAFTED HUMAN T-CELL ACUTE LYMPHOBlastic LEukemia

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Background. Despite major therapeutic improvements, a fraction of patients with acute lymphoblastic leukemia (ALL) still relapse and experience eventual refractory leukemia, pointing to the need for relevant preclinical models to design new therapeutic strategies. Genomic studies in human ALL have revealed intra-clonal heterogeneity at diagnosis, which causes B-ALL in Tg mice. The phenotype of the tumor seen in the Tg mice was similar to those of human disease. MiR125b binds to trp53inp1 and its overexpression is suggested to confer anti-apoptotic characteristics on cells. Overexpression of miR125b might be associated to BCP-ALL derived from BCR/ABL hematopoietic clone.

0490 CLONAL SELECTION IN XENOGRAFTED HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA RECAPITULATES GAIN OF MALIGnANCY AT RELAPSE

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Results. For 8 patients, relapse samples could also be analyzed. Large scale gene-expression profiling, lentiviral-mediated shRNA knockdown in patient’s primary leukemic cells followed by competitive in vitro experiments, and in vitro assays for drugs response were performed to analyze functional features of clonal selection in xenograft leukemia. Results. We show that leukemia in recipient mice frequently initiated from minor subclones pre-existing at diagnosis and bearing additional genomic lesions of human cancer genes like PTEN, MYC, MYB, WT1, CDKN2A, and NOTCH1, reminiscent of clonal selection towards relapse in patients. Gene-expression profiling identified a robust signature of cell cycle and mitosis in the xenograft leukemia samples compared with the corresponding diagnosis samples. Importantly, Gene Set Enrichment Analysis (GSEA) found that this signature was also highly enriched in the cells at relapse in two independent series of human ALLs. Mimicking the effect of an additional genomic lesion in patient’s primary leukemia cells by shRNA-mediated knock-down conferred a selective advantage in competitive engraftment experiments, demonstrating that these lesions can be drivers of increased leukemia-initiating activity. Finally, xenograft leukemia cells had an overall diminished sensitivity to glucocorticoids and gamma-secretase inhibitor.

0491 MUTATION OF THE HEDGEHOG PATHWAY IN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

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SUMMARY/CONCLUSIONS. The Hedgehog (HH) signaling pathway plays an important role in cell growth and differentiation and is involved in the development of many tumors, including T cell development. Based on this important function, it is not surprising that several human diseases are caused by mutations in components of the HH pathway. Many studies have shown deregulation of this pathway in basal cell carcinoma, medulloblastoma, and other cancers. Also in chronic myeloid leukemia stem cells, activation of the HH pathway was suggested, but mutation in HH pathway components have not been identified in hematological malignancies. Aims. Upon mutation analysis of 97 candidate oncogenes in T-cell acute lymphoblastic leukemia (T-ALL), we identified PTCH1 (patched) mutations in several T-ALL cell lines (see abstract by V. Gianfelici et al.). The aim of this project was to confirm the involvement of the HH pathway in the pathogenesis of T-ALL and to determine the sensitivity of T-ALL cell lines to HH pathway inhibitors. Methods. We performed a mutagenic analysis of the different HH components in 17 T-ALL cell lines and 57 T-ALL patients and identified a set of T-ALL cell lines with 4 different HH antagonists (Cyclosporine, GDC-0449, GANT61 and Itraconazole) and tested the effect on proliferation, cell cycle and apoptosis. Results. Sequence analysis of PTCH1, PTCH2, SMG, SUFU, GLI1 and GLI3 revealed a number of mutations in at least one of these genes in 9/17 (53 %) cell lines and 9/57 (15%) primary T-ALL samples. In agreement with this, T-ALL cell lines showed sensitivity to HH pathway antagonists. Itraconazole, a natural antifungal triazole, that was recently discovered as a new inhibitor of the HH pathway was identified as the most potent HH inhibitor in T-ALL cell lines with IC50 values below 500 nM. Apart from a reduction of the proliferation, it also induced apoptosis and blocked the cell cycle. Conclusion. Our data demonstrate an important role of the HH pathway in the pathogenesis of T-ALL, and show potent inhibitory activity of HH pathway inhibitors on T-ALL cell proliferation and survival.

0492 THE ERYTHROPOIETIN RECEPTOR (EPO) IS Deregulated by ETv6-RuN1X (TEL-AML1) in EARLY B LINEAGE PROGENITOR CELLS

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Background. ETV6-RUN1X (TEL-AML1) fusion is a pre-natal and initiating event in childhood acute lymphoblastic leukaemia (ALL). The erythropoietin receptor gene (EPO) is consistently highly expressed ectopically in TEL-AML1+ ALL but any role in leukemogenesis driven by TEL-AML1 remains to be confirmed. Aims. To assess the impact of TEL-AML1 expression on candidate ‘pre-leukaemic’ stem cells and to demonstrate the presence of functional, ligand binding EPO on TEL-AML1+ ALL cells that may provide these cells with a survival signal. Methods. Biotinylated erythropoietin (EPO) and flow analysis were used to assess cell surface EPO receptor levels. Quantitative PCR, ChIP, luciferase reporter assays and EMSA showed that TEL-AML1 directly regulates EPO. Standard tissue culture techniques were used to show cell survival in the presence of EPO alone and western blot to assess the mechanism of cell signalling. Results. Biotinylated EPO and flow analysis showed that the pre-B ALL TEL-AML1+ cell line REH has higher levels of EPO than similar non-TEL-AML1 cell lines. A “blind screen” of CD19+ cells isolated from 10 patients with pre-B ALL, identified five patients with high expression of ligand-binding EPO, four of which were subsequently identified as TEL-AML1+. The inducible
expression of TEL-AML1 in lymphoid BaF3 cells, or its constitutive expression in a murine transplant model was sufficient to increase expression of EPO. EMSA and ChIP experiments within the EPO promoter confirmed occupancy of AML1 consensus binding sites by TEL-AML1 and luciferase reporter assays in the presence of the fusion protein showed up-regulation of EPO promoter activity. Given the proposed pro-survival properties of EPO on non-erythroid cells, we asked if the observed increase in expression of the EPO could correlate with increased cell survival in the presence of EPO. Cell survival experiments including growth curves, propidium iodide staining and analysis of anti-apoptotic gene markers revealed that IL3-dependent cells expressing TEL-AML1 showed a prolonged survival in the presence of EPO. However, turning off TEL-AML1 in these cells resulted in cell death even in the presence of EPO, suggesting that this effect is a consequence of TEL-AML1 expression alone. Signalling through EPO in the presence of EPO was confirmed by phosphorylation analysis of Jak and Akt pathways, analysis of STAT5 activity and the concomitant up-regulation of Bcl-2. EPO functionality in the presence of EPO was also demonstrated in TEL-AML1+ patient cells. In our model of human pre-leukemia, normal human CD34+ cord blood cells were transduced in vitro with a lentivirus capable of expressing both TEL-AML1 and GFP and cells were ‘primed’ for pre-B lineage commitment. These TEL-AML1+ cells also showed increased levels of functional cell surface EPO receptor, elevated survival gene targets - again supporting a role for enhanced cell survival through the EPO-EPOR axis. Summary: These data support the contention that TEL-AML1 directly activates ectopic expression of a functional EPOR, which provides cell survival signals that contribute critically to persistence of the pre-malignant clone in patients.

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MICRORNA SIGNATURES IN NORMAL AND MALIGNANT T-CELL DEVELOPMENT

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Background. T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy of thymocytes. In order to comprehensively assess the role of miRNAs (miRNAs) in T-ALL, we compared miRNA action in T-ALL cell lines. Cross-comparison with the miRNA library screen allowed the identification of five T-ALL promoting miRNAs (miR-19b, miR-20a, miR-20b, miR-92, miR-142-3p, miR-150, miR-100, miR-26a, miR-16 and miR-342). High expression of this subset of 10 miRNAs was confirmed in a series of 18 T-ALL cell lines. Cross-comparison with the miRNA library screen allowed the identification of five T-ALL promoting miRNAs (miR-19b, miR-20a, miR-26a, miR-92 and miR-223). Remarkably, these miRNAs produce overlapping and cooperative effects on validated target genes with known tumor suppressor function in T-ALL, including IKAROS (IKZF1), PTEN, BIM, PHF6, NF1 and FBXW7. In addition, we compared miRNA expression patterns in 50 human T-ALL samples and 5 distinct subsets of normal developing T-cell progenitors with an unbiased miRNA library screen followed by computational target identification and functional assessment of the most relevant candidate miRNAs in a murine T-ALL model. Methods. Using high-throughput quantitative stem-loop RT-PCR, 450 miRNAs were profiled in a T-ALL patient cohort including 12 HOXA, 15 TAL/LMO, 10 TLX3 and 5 TLX1 rearranged patient samples as well as in 5 different subsets of sorted T-cell populations from human thymus. An unbiased miRNA library screen was performed in myc-transduced MEFs, based upon rescue for myc-induced apoptosis, followed by validation for individual miRNAs in FLS-12 lymphocytes and in vitro NOTCH1-sensitized murine T-ALL model. Results. A total of ten miRNAs were highly expressed in the entire cohort of T-ALLs, i.e. miR-223, miR-19b, miR-20a, miR-92, miR-142-3p, miR-150, miR-93, miR-26a, miR-16 and miR-342. High expression of this subset of 10 miRNAs was confirmed in a series of 18 T-ALL cell lines. Cross-comparison with the miRNA library screen allowed the identification of five T-ALL promoting miRNAs (miR-19b, miR-20a, miR-26a, miR-92 and miR-223). Remarkably, these miRNAs produce overlapping and cooperative effects on validated target genes with known tumor suppressor function in T-ALL, including IKAROS (IKZF1), PTEN, BIM, PHF6, NF1 and FBXW7.

Conclusion. A comprehensive and unbiased analysis of miRNA action in T-ALL and normal developing thymocytes reveals a cooperative role for a small set of miRNAs in suppression of key T-ALL suppressor genes, identifies miRNA signatures in genetic T-ALL subgroups and provides insights into miRNA controlled regulation of thymocyte maturation.
THE OPTIMAL DURATION OF ANTIICOAGULANT THERAPY IN PATIENTS WITH CANCER-RELATED DEEP VEIN THROMBOSIS: THE ADVANTAGE OF USING RESIDUAL VEIN THROMBOSIS (THE CANCER-DACUS STUDY)


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Type and duration of anticoagulation is still matter of debate in cancer patients with acute Deep Vein Thrombosis (DVT) of the lower limbs. Residual Vein Thrombosis (RVT) has been proven to be effective for assessing the optimal duration of oral anticoagulants in non cancer patients (Siragusa S et al Blood 2008:112:511-5). In the present study we evaluate the role of a RVT-based management of anticoagulation with Low-Molecular Weight Heparin in cancer patients with acute DVT.

Materials and Methods. Patients with active cancer and a first episode of DVT were treated with LMWH for 6 months (the first month at full dosage followed by dose reduction of 25% in the next 5 months). At the end of treatment, they were managed according to RVT findings: patients with RVT were randomized to continue anticoagulants for 6 additional months (Group A1) or to stop it (Group A2), while patients without RVT stopped LMWH (Group B). Outcomes were recurrent venous thromboembolism and/or major bleeding; patients were followed up for one year after LMWH discontinuation.

Results. Over a period of 56 months, 409 patients were evaluated; 62 were excluded (refusal, need for continuing anticoagulation, etc). In total, 347 were included in the study (Table 1). RVT was detected in 242 (69.7%) patients; recurrent events occurred in 3 cases (2.8%) (Table 2 and Figure 1). The adjusted Hazard Ratio (HR) for age and sex between RVT groups (Group A2 vs A1) was 1.58 (95% confidence interval [CI], 0.85-2.39; P = .145). The adjusted HR between group A1 versus RVT-negative group (B) was 4.54 (CI 2.3-6.6; P = .028). Five major bleeding events occurred in Group A1 and two events both in Group A2 and B (Table 2).

Conclusions. The Cancer DACUS is the first ever study evaluating an individual marker for assessing duration of anticoagulation in active cancer population. Final results of the study show that absence of RVT identifies a group of patients at low risk for recurrent thrombosis who can safely stop LMWH after 6 months.
**0497**

**THROMBOPROPHYLAXIS FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH LENALIDOMIDE-BASED REGIMENS: A RANDOMIZED PHASE III STUDY OF ASPIRIN VS ENOXAPARIN**

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**Aims.** The primary objective of the study was to assess the efficacy and safety of aspirin (ASA) or low-molecular weight heparin (LMWH) as thromboprophylaxis in newly diagnosed MM patients younger than 65 yrs during Rd induction and MPR consolidation. Primary end-points were incidence of venous thromboembolism (VTE), acute cardiovascular events, sudden death, major and minor bleeding. Methods. a total of 402 transplantation candidates received four 28-day cycles of lenalidomide (25 mg d 1-21) and low-dose dexamethasone (Rd) induction and subsequently randomized to receive consolidation with lenalidomide + melphalan + prednisone (MPR) or high dose melphalan (MEL200). In this substudy, we compared the efficacy and safety of aspirin (ASA) or low-molecular weight heparin (LMWH) as thromboprophylaxis in newly diagnosed MM patients younger than 65 yrs during Rd induction and MPR consolidation. Primary end-points were incidence of venous thromboembolism (VTE), acute cardiovascular events, sudden death, major and minor bleeding.

**Methods.** a total of 402 transplantation candidates received four 28-day cycles of lenalidomide (25 mg d 1-21) and low-dose dexamethasone (40 mg d 1,8,15,22) (Rd) as induction and randomized to consolidation with six 28-day cycles of melphalan (0,13 mg/kg days 1-4), prednisone (28 mg d 1-28) and lenalidomide (30 mg days 1-21) (N=202) or tandem melphalan 200 mg/mq days 1-4) and lenalidomide (10 mg days 1-21) (N=200) with stem-cell support (N=200). A total of 342 patients without clinical indication or contraindication for a specific anticoagulant were enrolled in this substudy and randomly assigned to receive Aspirin 100 mg/d (N=176) or Enoxaparin 40 mg/d (N=166) during induction with Rd and consolidation with MPR. Results. patient characteristics and distribution of major risk factors were similar in the two groups. During induction, the overall incidence of any 3-4 thrombotic events was 2,27% in the ASA group and 1,20% in the LMWH group (p=0.659). Deep vein thrombosis were equally distributed in the two groups (1,13% Vs 1,20%, p=0.466), while pulmonary embolism was observed only in the ASA group (1,70%). Compared with LMWH, the absolute risk difference was +0.5% (95%CI=2.2 to 3.5, p=0.605) in the ASA group. All thromboembolic events occur in early phase of treatment (median 1.3 months). Only 1% of minor bleeding was detected in the LMWH group and no cardiovascular events were observed. During MPR consolidation only one thrombotic event was seen in the LMWH group.

**Conclusion.** our data indicate a low overall incidence of thrombotic events in all groups. Both ASA and LMWH show similar safety and efficiency in reducing thromboembolic events in newly diagnosed multiple myeloma patients treated with lenalidomide-based regimens.

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**0498**

**TIMING OF TISSUE FACTOR (TF) MRNA AND HYPERCOAGULABILITY DOWNREGULATION BY ALL-TRANS-RETINOIC ACID (ATRA) IN ACUTE PROMYELOCYTIC LEUKEMIA (APL)**

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**Background.** Among the mechanisms responsible for intravascular clotting activation in APL, a key role is played by the procoagulant activities, including TF expressed by the promyeolocytic blasts. Differentiating therapy with ATRA or Arsenic Trioxide (ATO) achieves 90% complete remission rate and corrects the hyperactivity of the coagulation and fibrinolytic systems. The beneficial effects on the coagulopathy are at least in part due to the capacity of ATRA and ATO to downregulate TF expression in APL cells. However, it is not known whether the persistence of TF mRNA expression in peripheral APL cells might be a marker of hypercoagulability in these patients. Aim. In this study we evaluated the levels of TF mRNA in peripheral mononuclear cells (PBMC) obtained from 9 APL patients: 4 treated with ATRA+Idarubicin (IDA) and 5 treated with ATRA+ATO. Results. of TF mRNA were correlated to the plasma levels of thrombin antithrombin complexes (TAT), as an index of thrombin formation and inhibition, and of activated factor VII-Antithrombin complex (FVIIa-AT), as an index of TF exposure and inhibition on blast cells. Five healthy subjects acted as the control group. Methods. Blood samples were obtained at the onset of the disease (T0) and after 7 (T1), 14 (T2) and 28 (T3) days of treatment. PBMC were isolated from whole blood and TF mRNA was assessed using real time-polymerase chain reaction. Results were normalized versus T0. Platelet-free plasma was obtained by two serial centrifugations (both 4,000rpm for 15 min) of citrated whole blood. ELISA methods were used for the measurement of plasma levels of TAT (Siemens) and FVIIa-AT complexes (STAGO). Results. The levels of TF mRNA of APL PBMC were significantly elevated at the onset of the disease compared to controls (p<0.05). In patients treated with ATRA+IDA the levels of TF mRNA decreased by 68% at T1, by 70% at T2 and by 90% at T3; similar reductions were observed in patients treated with ATRA+ATO, i.e. 64% at T1, 53% at T2 and 82% at T3. Plasma concentration of TAT complex of APL patients significantly decreased (p<0.05) from T0 (48.9±6.43 mg/L) to T3 (8.18±0.92 mg/L). Similarly, plasma concentration of FVIIa-AT significantly decreased from T0 (287.10±36.51 pm) to T3 (168.51±16.62 pm). The statistical analysis revealed significant correlations between TF mRNA and TAT at T1 and T3 (ATRA+IDA: T1 R2=0.691; T3 R2=0.927; ATRA+ATO: T1 R2=0.685) and between TF mRNA and FVIIa-AT at T1 and T2 (ATRA+IDA: T1 R2=0.767; T2 R2=0.672; ATRA+ATO T1 R2=0.828; T2 R2=0.817). Summary/Conclusions. Our results show that TF expression is elevated in PBMC of APL patients at the onset of the disease and is downregulated either by ATRA+IDA or ATRA+ATO treatment following a similar pattern of reduction. The significant correlation between TF mRNA reduction and the decrease of the two markers of hemostatic system activation (TAT and FVIIa-AT) suggests that TF mRNA as a useful surrogate marker of hypercoagulability in multicenter studies.
Experimental stem cell transplantation

REGULATION OF ACUTE GRAFT VERSUS HOST DISEASE BY MICRONRNAS

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Background. Acute Graft-Versus-Host disease (aGVHD) is a frequent complication of allogeneic bone marrow transplantation (BMT) in which donor T cells destroy HLA mismatched host tissues. Recent studies indicate that microRNA (miR)-155 is induced upon CD4+ cell activation and promotes Th1 differentiation. Aims. Based on the importance of T cells in aGVHD pathogenesis and the regulation of immune responses by miR-155, here we propose to investigate whether miR-155 expression is deregulated in donor T cells during aGVHD and whether miR-155 is involved in the modulation of this process. Methods. We used a MHC mismatched BMT aGVHD stimulation, and we measured miR-155 expression by qRT-PCR. Results. CD4+CD62L- effector cells were isolated from spleen cells (20X10⁶) and T cell depleted bone marrow (t-BM;5X10⁶) from C57BL/6 (B6) donors were transferred i.v. into lethally irradiated B6D2F1 recipient mice. Control groups included mice that received t-BM only or received no cell infusion. CD4+CD62L- cells were isolated from mice with aGVHD exhibited increased miR-155 expression with respect to the same cell populations obtained from the t-BM only group (4 fold increase, p<0.001). To confirm that a causal relationship exists between miR-155 and aGVHD severity, we repeated the MHC mismatched murine experiment using B6 mice deficient for miR-155 or control B6 mice. Mice receiving donor spleen cells from B6/miR-155 KO mice exhibited dramatically lower mean GVHD scores and improved survival compared to those receiving WT spleen cells (87% vs. 13% of mice alive at 70 days, respectively; p<0.001). GVHD histological scores in the spleen, liver or gut were remarkably lower in recipients from miR-155 KO (p<0.005). Overall survival, GVHD scores and histological GVHD findings were similar between miR-155 KO and WT t-BM only group. Mice receiving miR-155 KO spleen cells also had significantly lower TNF-α levels than WT controls (14 pg/ml vs. 48 pg/ml, p=0.005). To further establish the regulatory role of miR-155 in aGVHD, we generated transgenic mice that over-expresses miR-155 in T cells under the LCK promoter (B6 LCK-miR-155). Using splenocytes from LCK-miR-155 TG mice we performed the mismatched MHC experiments as described previously. Recipients of miR-155 over-expressing splenocytes developed hyper-acute GVHD (confirmed by pathology) and died shortly after transplant (within 2-3 weeks), while recipients of WT cells developed lethal aGVHD significantly later (p=0.05). Relevant to human aGVHD, we measured miR-155 expression in the colon tissues of aGVHD patients (n=5) or controls (n=4) using DIG-tagged anti-miR-155 LNA and found a dramatic up-regulation of miR-155 in the mucosa of aGVHD patients, while it was negative in healthy controls. Finally, we performed the B6 into F1 MHC-mismatched transplants as described and treated 6 mice with antisense miR-155 (LNA anti-155) and 6 mice with a scrambled control (n=6) 5 mg/kg twice a week (i.v) 2 weeks starting at day +7. The mice that received LNA anti-155 showed higher survival rate compared to mice that received the scrambled control (p=0.05). Conclusions. Collectively, our data indicate that miR-155 modulates aGVHD, and thus point to miR-155 as a potential target for therapeutic intervention for aGVHD.

0500

IKAROS-NOTCH SIGNALING IN HOST ANTIGEN PRESENTING CELLS REGULATES EXPERIMENTAL GRAFT-VERSUS-HOST DISEASE (GVHD) AND GRAFT-VERSUS-LEUKEMIA (GVL) RESPONSES

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Background. Host antigen presenting cells (APCs) are necessary for induction of graft-versus-host disease (GVHD) responses. But the APC autonomous molecular mechanisms that are critical for modulation of GVH are not known. Because Ikaros (Ik) is known to negatively regulate certain dendritic cell (DC) responses (the most potent APCs), we hypothesized that its deficiency in host APCs will reduce GVHD. Methods and Results. We generated [B6[ARRROWRIGHT]B6] and [Ik/-[B6[ARRROWRIGHT]B6] chimeras and utilized them as recipients in C3H/SW B6 model of acute GVHD. The [Ik/-[B6[ARRROWRIGHT]B6] animals showed significantly worse survival, GVHD specific clinical severity and histopathological damage than the allogeneic [B6[ARRROWRIGHT]B6] animals (p<0.001). In vitro, CFSE and annexin labelling studies demonstrated that Ik/-DCs caused greater proliferation without altering the rate of apoptosis. To characterize the molecular mechanisms we evaluated the role of putative molecular targets of Ikaros, the Notch signaling pathway. Ik/-DCs, at steady state, showed an increase in the expression of several Notch target genes such as Hes-1, Hes-5, etc. Blockade of Notch signaling with γ-secretase inhibitor (DAPT) mitigated the enhanced allo-stimulatory capacity of the Ik/-DCs in vitro and decrease donor T cell expansion and improved body weight loss in vivo. We next hypothesized that given the enhanced GVHD response that was associated with increased proliferation and preserved cytotoxicity of allo-T cells, the GVL response will also be enhanced in the recipients with Ik/-APCs. Unexpectedly, the [B6[ARRROWRIGHT]B6] and [Ik/-[B6[ARRROWRIGHT]B6] chimeras when transplanted with tumor cells demonstrated equivalent GVL responses despite greater severity of GVHD. Conclusions. Together our data demonstrate differential regulation of GVHD and GVL at the level of host APCs and show a role for a novel molecular pathway, the Ik-Notch axis, in the host APCs as an important modulator of GVH responses.

0501

ANALYSIS OF MiRNA EXPRESSION PROFILE AFTER HAEmatopoietic STEM CELL TRANSPLANTATION: A PROMISING TOOL FOR PREDICTING ACUTE GVHD

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Background. Allogeneic haematopoietic-stem-cell transplantation (HSCT) is the treatment of choice for many malignant and non-malignant disorders. Despite the recent advances in post-transplant immunosuppressive therapy, Graft-versus-Host Disease (GVHD) still represents the major life-threatening complication. Recent studies have indicated that microRNAs (miRNAs) circulate in a stable, cell-free form in the bloodstream and that the abundance of specific miRNAs in plasma or serum can serve as biomarkers of cancer and other diseases. Aim. This study was aimed at the prospective analysis of miRNA expression profile in the plasma of allo-transplanted patients in order to detect specific miRNAs with predictive role for acute GVHD (aGVHD). Methods. After informed consent, we collected plasma samples from 10 healthy donors and 22 patients (median age: 59 and 41 years) who received unmanipulated HSCT (18 from Matched Unrelated Donors and 4 from HLA-matched siblings). Blood samples were collected weekly after HSCT and patients were monitored to assess aGVHD onset. Three of 22 patients developed intestinal GVHD (grade 3) while 9 of 22 patients developed cutaneous GVHD (grade 2-3). MicroRNAs were isolated from the plasma of patients using a modified mirVana® miRNA Isolation Kit (Ambion Inc). The miRNA expression profile was examined using a quantitative PCR-method (TaqMan® Human microRNA
Carda, Applied Biosystems) that allows the analysis of 384 human miRNAs by low density array technology. The results obtained were subsequently validated with specific miRNA Single Assays (Applied Biosystems). Hsa-miR-16 was used for data normalization due to its stability in all the samples analyzed. Relative quantification of miRNA expression was calculated with the 2-ΔΔCt method. Cluster analysis was performed using an agglomerative hierarchical algorithm with average linkage as a distance measure. Differential miRNA expression profile was investigated with the Mann-Whitney test. Results. Circulating miRNAs are detectable and amplified in all samples analyzed. Unsupervised hierarchical clustering of miRNAs present before the onset of GVHD, showed that specific circulating miRNA expression signatures exist between patients who will develop GVHD from those who will not. By comparing the miRNA expression profiles of GVHD patients and non-GVHD patients, we identified a group of 13 miRNAs upregulated in the plasma of GVHD patients (p<0.05). We then aimed at assessing whether this 13-miRNA panel provided information regarding the involvement of specific target organs. MiR-194 and miR-367 are overexpressed in patients developing intestinal GVHD (p<0.05). Of interest, both miRNAs are implicated in the differentiation of the gastrointestinal epithelium. A significant upregulation of miR-203 was observed prior to the onset of cutaneous aGVHD (p<0.001). The involvement of miR-203 in cutaneous aGVHD is supported by previous papers demonstrating that this target is required for keratinocytes differentiation, and SOCS2, a protein involved in the pathogenesis of GVHD (Hill et al. Blood, 2010). Conclusions. Our results demonstrate that the analysis of miRNA expression profile after HSCT is a promising tool for predicting aGVHD onset. Significantly higher plasma levels of miR-203 characterize patients who will develop skin-only GVHD whereas an upregulation of plasma levels of miR-194 and miR-367 may be used to predict the risk of developing intestinal aGVHD.

0502
IN VITRO-ESTABLISHED ALLOANTIGEN-SPECIFIC CD8+ CTLS MEDIATE GRAFT-VERSUS-TUMOR ACTIVITY IN THE ABSENCE OF GRAFT-VERSUS-HOST DISEASE

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Background. Allogeneic bone marrow transplantation (BMT) is a curative treatment modality for hematopoietic malignancies such as acute and chronic leukemias and lymphomas. Mature donor T cells in the allograft support engraftment, promote early T cell immunity of the recipient and mediate the graft-versus-leukemia (GVL) effect. However, these donor T cells are also responsible for the induction of graft-versus-host disease (GVHD) by attacking recipient tissue such as liver, skin and gastrointestinal epithelium. Aims. In our study aimed at the analysis of the CMV-specific as well as allo-HLA-reactivity through the repeated transfer of polyclonal donor T cells. This procedure resulted in transient expression of the in vitro transcribed TCR for up to one week. To compare different T-cell populations with regard to their alloreactive potential, we generated TCRp65 transfected naive and memory T-cell subsets. The latter have been reported to induce less alloreactivity due to a more restricted endogenous TCR repertoire. Although naive and memory T-cell subsets showed comparable expression of TCRp65, memory CD8+ T cells mediated superior cytotoxicity and IFN-γ production against CMV-infected fibroblasts for up to one week. Alternatively, we generated EBV/HLA-A*0201 peptide-specific T-cell lines and transfected them with TCRp65 RNA to obtain EBV/CMV-bispecific T cells. As with TCR redirected memory T-cell subsets, EBV/CMV-bispecific CD8+ T cells showed strong reactivity against CMV-infected fibroblasts for up to one week without hampering the endogenous EBV peptide-specific effector function. To analyze the allo-HLA-reactivity of the naive, memory and EBV-specific T-cell populations, we assayed their IFN-γ-secretion upon stimulation with different HLA-mismatched donor EBV-transformed B cells (EBV-LCL) as well as CD40 ligand activated B-cells. Although we tested only a small panel of 6 HLA-mismatched donors, alloreactivity was solely mediated by T-cell populations of naive phenotype. Moreover, no differences were obtained with either TCRp65 RNA transfected or untransfected T cells, assuming that mixed dimer formation between introduced and naturally expressed TCR chains did not induce additional allo-HLA-reactivity in our studies. Summary. Our data demonstrate that memory T-cell populations from CMV-negative donors can be easily redirected with TCRp65 RNA, thereby gaining CMV-specific T-cell effector function for a considerable time period. Due to their decreased alloreactivity, we believe that TCRp65 RNA redirected memory T-cell populations have the potential to be further developed as a therapeutic ‘off-the-shelf’ reagent for CMV-positive patients who undergo allogeneic HSCT from CMV-negative donors.

0503
LOOKING FOR MOST SUITABLE CELLS FOR T-CELL RECEPTOR RNA TRANSFER - MEMORY T CELLS OFFER CONSIDERABLE ADVANTAGES

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Background. Reactivation of latent Cytomegalovirus (CMV) infection is a common and sometimes life-threatening complication following allogeneic hematopoietic stem cell transplantation (HSCT). Long-term virus control requires the re-establishment of protective antiviral T-cell immunity in the host. The latter is challenging, particularly if the donor is CMV-negative and thus, no CMV-reactive T cells are being transferred. Hence, the graft-versus-host disease (GVHD) induced by donor T cells may negatively influence HSCT results. To overcome the limitations of vector-based gene transfer that hamper clinical translation, we used in vitro transcribed RNA encoding CMV-specific TCR for electroporation of non-reactive HLA-A2+ T cells. This procedure resulted in transient expression of the in vitro transcribed TCR for up to one week. To compare different T-cell populations with regard to their alloreactive potential, we generated TCRp65 transfected naive and memory T-cell subsets. The latter have been reported to induce less alloreactivity due to a more restricted endogenous TCR repertoire. Although naive and memory T-cell subsets showed comparable expression of TCRp65, memory CD8+ T cells mediated superior cytotoxicity and IFN-γ production against CMV-infected fibroblasts for up to one week. Alternatively, we generated EBV/HLA-A*0201 peptide-specific T-cell lines and transfected them with TCRp65 RNA to obtain EBV/CMV-bispecific T cells. As with TCR redirected memory T-cell subsets, EBV/CMV-bispecific CD8+ T cells showed strong reactivity against CMV-infected fibroblasts for up to one week without hampering the endogenous EBV peptide-specific effector function. To analyze the allo-HLA-reactivity of the naive, memory and EBV-specific T-cell populations, we assayed their IFN-γ-secretion upon stimulation with different HLA-mismatched donor EBV-transformed B cells (EBV-LCL) as well as CD40 ligand activated B-cells. Although we tested only a small panel of 6 HLA-mismatched donors, alloreactivity was solely mediated by T-cell populations of naive phenotype. Moreover, no differences were obtained with either TCRp65 RNA transfected or untransfected T cells, assuming that mixed dimer formation between introduced and naturally expressed TCR chains did not induce additional allo-HLA-reactivity in our studies. Summary. Our data demonstrate that memory T-cell populations from CMV-negative donors can be easily redirected with TCRp65 RNA, thereby gaining CMV-specific T-cell effector function for a considerable time period. Due to their decreased alloreactivity, we believe that TCRp65 RNA redirected memory T-cell populations have the potential to be further developed as a therapeutic ‘off-the-shelf’ reagent for CMV-positive patients who undergo allogeneic HSCT from CMV-negative donors.

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**Results of COMFORT-I, a Randomized, Double-Blind Phase III Trial of the Jak1 and Jak2 Inhibitor Ruxolitinib (INCBO18424) versus Placebo for Patients with Myelofibrosis**

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**Background.** R-CHOP induction is considered the standard regimen for elderly patients with mantle cell lymphoma (MCL), but remissions are of short duration. Maintenance with interferon-alfa has been suggested to be effective, but side effects were serious. Rituximab seemed a promising candidate for improvement. Aims. In the European MCL Elderly trial we studied different induction regimens as well as the role of maintenance therapy. Here, the results of the maintenance are reported. Methods. Eight countries participated in this trial. Patients >60 yrs not eligible for high dose therapy with stage II-IV MCL were included. Initially, patients were randomized between 8 cycles of 3-weekly R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednison) or 6 cycles of 4-weekly R-FC (rituximab, fludarabine, cyclophosphamide). Subsequently, patients in complete or partial remission (CR, CRunconfirmed or PR) underwent a second randomization between maintenance with rituximab 275 mg/m² every 2 months or interferon-alfa 2a or 2b (IFN) (regular IFN weekly 3×3 MIU or pegylated IFN 1×1 µg/kg). Second randomization was stratified for cause of disease (MFSAF v2.0, and survival. Additional exploratory endpoints included change in quality of life (QoL) measured by the EORTC-QLQ-C30, fatigue measured with the PROMIS Fatigue Scale, molecular and serum biomarkers, and transfusion dependence. Results. 309 patients were randomized: 155 to ruxolitinib and 154 to placebo. Median follow-up was 32.2 weeks. The proportion of patients with ≥5% reduction in spleen volume at week 24 of therapy, assessed by blinded central review of spleen MRI or CT. Secondary endpoints were change in symptom score over time, and all showed improvement relative to placebo. At week 24, the proportion of patients with ≥5% improvement in symptom score was 45.9% vs 5.5% (ruxolitinib vs placebo, p < 0.0001) and the mean percent change in total symptom score was an improvement of 46.1% vs a worsening of 41.8% (ruxolitinib vs placebo, p < 0.0001). There were 10 vs 14 deaths (ruxolitinib vs placebo, HR 0.67, p = 0.33).

**Discussion.** Ruxolitinib is a selective JAK1 and JAK2 inhibitor with demonstrated clinical activity in myelofibrosis. Asymptomatic patients were randomized to placebo or ruxolitinib at a dose of 15 or 20 mg PO BID depending on baseline platelet count (100-500 × 10³/µl or >500 × 10³/µl, respectively). The dose was optimized for efficacy and safety during treatment. The primary endpoint was the proportion of patients with ≥35% reduction in spleen volume at week 24, assessed by blinded central review of spleen MRI or CT. Secondary endpoints were duration of response and time to response. Treatment-related adverse events (AEs) were similar between groups, with 23% vs 21% in any grade seen in >20% of patients on either arm of the study were rash (17.9% vs 11.7%), diarrhea (13.9% vs 29.0%), fatigue (23.2% vs 29.0%), and peripheral edema (23.7% vs 11.7%). AEs were most common in patients with anemia (51% vs 19.3%), thrombocytopenia (25.2% vs 33.3%), and peripheral edema (18.7% vs 22.5%). The most common AEs of grade 3 or higher were anemia (10.3% vs 3.3%), thrombocytopenia (54.2% vs 9.3%), fatigue (25.2% vs 33.3%), and peripheral edema (18.7% vs 22.5%).
marked clinical benefits in spleen size, debilitating symptoms, and QoL that were rapid in onset and sustained. Anemia and thrombocytopenia were among the most common AEs but they were manageable, as demonstrated by the low withdrawal rate due to these events. The overall safety profile relative to placebo in myelofibrosis was acceptable.

**0506**

**DYNAMIC MUTATION PROFILES OF LEUKAEMIC CELL POPULATIONS IN RESPONSE TO TREATMENT ARE REVEALED BY GENOME-WIDE SEQUENCING OF SEQUENTIAL SAMPLES FROM PATIENTS WITH B-CLL**

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B-cell chronic lymphocytic leukaemia (B-CLL) is the most common leukaemia in the Western World. It is characterized by clinical and biological heterogeneity and a chronic relapsing course making it an ideal model to study the molecular events underlying relapse and the development of treatment resistance. Whole genome sequencing (WGS) has the potential to identify molecular pathways perturbed in cancer. However, the clinical significance of this information is not clear. Our aim was therefore to (1) establish the molecular profiles of sequential samples from patients with B-CLL at every relapse using WGS and (2) correlate findings with clinical outcome. Patients undergoing multiple rounds of treatment including second-generation monoclonal antibody treatment were selected for study. WGS was carried out to an average depth of >30-fold depth on each of five sequential peripheral blood samples per patient plus matched germline buccal swab controls. Sequencing employed SBSv5 chemistry on the HiSeq2000 instrument. Paired 100-base reads were aligned to the human reference GRCh37.1/hg19 and candidate single nucleotide variants (SNVs), insertions, deletions and copy number variants (CNVs) were detected in all genomes. Candidate somatic variants were called in each tumour sample by subtraction of all germ-line variants (i.e. those present in the matched normal sample for the individual). Somatic variants were clustered based on the profiles of allele frequency along the time-course of the entire tumour set within an individual. Targeted deep sequencing (TDS) of amplicons containing selected non-synonymous SNVs was carried out to a depth of ~10,50,000-fold and mutant allele frequencies were calculated by establishing the fraction of reads containing the mutant allele. An average of 4,600 mutations per sample were identified in each patient. On average, just 10 mutations were non-synonymous, non-coding or frameshift mutations. On the basis of their varying mutation frequency profiles over time, the mutations could be clustered into up to 4 classes depending on patient: (I) those that disappear after treatment with purine analogues; (II) those that are present throughout the course of the disease and therefore resistant to treatment; (III) those that are initially detected at low frequency and then expand (Class III); and (IV) those not detected until later stages. So far, the data suggest that Class I and Class II sub-clones are characterized by pathways regulating innate immune responses and apoptosis (eg ADAD1, SAMHD1, BCL2L13) whereas Class III and IV sub-clones carry mutations in general cancer pathways (eg MEK1, ASXL1, FAT3, NRG3). **Conclusions.** This is the first sequential WGS analysis of B-CLL samples. We reveal that the molecular composition of this leukaemia alters with treatment. We identify mutations that are eradicated by or resistant to treatment including mutations important in regulation of innate immune response and cancer progression. These findings and the results of ongoing studies of other B-CLL cases will direct future clinical trials and therapeutic decisions.

**0507**

**SILENCING OF RHOA NUCLEOTIDE EXCHANGE FACTOR, ARHGEF3 REVEALS ITS UNEXPECTED ROLE IN IRON UPTAKE**

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**Background.** Genome-wide association meta-analysis studies (GWAS) have identified over 100 independent genetic loci associated with blood cell indices, including volume and count of platelets and erythrocytes. **Aims.** Although several of these loci encode known regulators of haematopoiesis, the mechanism by which the majority of sequence variants exert their effect on blood cell formation remains elusive. **Methods.** A recent meta-analysis of the GWAS in 68,000 individuals of European ancestry has identified 68 genomic loci (53 new) associated with platelet count and volume. Here we demonstrate a functional validation for five of these novel loci in Danio rerio by the means of gene silencing. **Results.** Our experiments in Tg(cd41:EGFP) transgenic fish reveal important functions for genes encoding guanine nucleotide exchange factors (arhgef3), tropomyosins (tpm) and poorly characterized transcriptional regulators (mft145 and mjt14c), in regulating thrombopoiesis. In addition, lineage relatedness of megakaryocytes and erythrocytes prompted us to explore the putative role of these genes in erythropoiesis and silencing of all five genes but tpm (transcription of its human homologue TPM1 in blood is restricted to megakaryocytes) also exerted an effect on primitive erythropoiesis. The silencing of arhgef3 on erythropoiesis was the most profound and this prompted further detailed studies. Examination of peripheral blood from arhgef3 morpholino (MO) injected zebrafish embryos revealed that arhgef3-depleted erythroid cells, once provided with intracellular iron are fully capable of haemoglobinisation. Disruption of the arhgef3 target, RhoA, also produced severe anaemia, which was again rescued by iron injection. Moreover, silencing of the ARHGEF3-expressing line, the erythromegakaryoblastoid cell line K562 revealed that the uptake of transferrin, the main blood plasma protein for iron transport, was severely impaired. **Conclusions.** Taken together, this is the first study to provide evidence for ARHGEF3 being a regulator of transferrin and iron uptake in erythroid cells, through activation of RhoA. Taken together, our findings demonstrate the value of pursuing GWAS signals as a new and exciting forward genetics approach in identifying novel regulatory molecules and signalling pathways in haematopoiesis.

**0508**

**MELPHALAN/PREDNISONE/LENALIDOMIDE (MPR) VERSUS HIGH-DOSE MELPHALAN AND AUTOLOGOUS TRANSPLANTATION (MEL200) IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS: A PHASE III STUDY**

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newly diagnosed MM patients younger than 65 years.

The introduction of novel agents questions the role of autologous stem-cell transplantation (ASCT).

BRAF MUTATIONS IN HAIRY CELL LEUKEMIA


Background. Hairy cell leukemia (HCL) is a well defined clinico-pathological entity whose underlying genetic lesion is still obscure. We searched for HCL-associated mutations by massively parallel sequencing of the whole exome of leukemic and matched normal mononuclear cells purified from the peripheral blood of one patient with HCL. Whole exome sequencing identified 5 missense somatic clonal mutations that were confirmed at Sanger sequencing, including a heterozygous V600E mutation involving the BRAF gene. Since the BRAF V600E mutation is oncogenic in other tumors, further analyses were focused on this genetic lesion. Sanger sequencing detected mutated BRAF in 46/46 additional HCL patients (47/47 including the index case; 100%). None of the 193 peripheral B-cell lymphomas/leukemias other than HCL that were investigated carried the BRAF V600E mutation, including 36 cases of splenic marginal zone lymphomas and unclassifiable splenic lymphomas/leukemias. Immunohistological and Western blot studies showed that HCL cells express phospho-MEK and phospho-ERK (the downstream targets of the BRAF kinase), indicating a constitutive activation of the RAF-MEK-ERK mitogen-activated protein kinase pathway in HCL. In vitro incubation of BRAF-mutated primary leukemic cells from 5 HCL patients with PLX-4720, a specific inhibitor of active BRAF, led to marked decrease of phosphorylated ERK and MEK. This finding may have relevant implications for the pathogenesis, diagnosis and targeted therapy of this hematological disorder (Funded by the Associazione Italiana Ricerca Cancro and others).
First line therapy in Multiple Myeloma

0510
BORTEZOMIB-THALIDOMIDE-DEXAMETHASONE COMPARED WITH THALIDOMIDE-DEXAMETHASONE AS CONSOLIDATION THERAPY AFTER DOUBLE AUTOLOGOUS STEM-CELL TRANSPLANTATION IN NEWLY DIAGNOSED MULTIPLE MYELOMA

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We prospectively compared thalidomide-dexamethasone (TD) with bortezomib plus TD (VTD) as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation (ASCT) in patients with newly diagnosed multiple myeloma (MM). 474 patients randomized to the VTD (n=236) or TD (n=238) arm were analyzed on an intention-to-treat basis. After three 21-d cycles of induction therapy, the rate of complete response (CR) plus near CR (nCR), the primary study endpoint, was threefold higher with VTD compared to TD (31% vs 11%, respectively; p<0.0001). Post-ASCT consolidation therapy comprised two 35-d cycles of either VTD (V, 1.3 mg/m2 once-weekly; T, 100 mg/d through d 1 to 70; D, 320 mg/cycle) or TD (same doses as in VTD), according to the initial randomization. After consolidation therapy, the rate of CR-nCR, a secondary study endpoint, was 62% in the VTD and 45% in the TD arm (p=0.0002). A per-protocol analysis of 323 patients who actually received VTD (n=161) or TD consolidation therapy (n=162) was performed to evaluate the activity and toxicities of the two regimens. McNemar test results showed that VTD consolidation significantly increased the rate of CR (p=0.005) and CR-nCR (p=0.01), an objective failed by TD consolidation (p=0.07 and p=0.2 for upgraded CR and CR-nCR rates, respectively). Overall, the probability to upgrade high-quality response from less than CR before consolidation to CR after consolidation was two-fold higher with VTD compared to TD (11% vs 6%, respectively). Non hematologic grade 3-4 adverse events were 11% with VTD and 10% with TD, including peripheral neuropathy (1% vs 0%, respectively) and skin rash (0.6% in each of the two treatment arms). Dose adherence of study drugs was very close to that planned; in particular, patients in the VTD arm received 97% of planned doses of bortezomib and thalidomide, while the corresponding value for thalidomide in the TD arm was 97%. In a substudy, post-consolidation molecular detection of minimal residual disease (MRD) was evaluated by means of patient-specific primers. VTD consolidation improved significantly the rate of molecular negativity, up to the 65% value (p=0.007 using the McNemar test), a benefit not seen with TD consolidation (48% of molecular negativity; p=0.06). Quantitative analysis of MRD after the 2 planned cycles of VTD consolidation therapy showed a 5-log reduction in residual clonal cells. By the opposite, consolidation therapy with TD yielded a 1 log reduction in MRD. Attainment of molecular negativity after consolidation therapy was a strong predictor of favourable clinical outcomes. In particular, the 3-year estimate of progression-free survival was as high as 89% for patients with undetectable MRD after consolidation therapy, while the corresponding value for patients with molecular positivity was 47% (p=0.04). It is concluded that VTD consolidation was more effective than TD in upgrading the rate of high-quality responses and yielding unprecedented high rate of molecular remission, in excess of 60%. Achievement of molecular negativity favourably affected clinical outcomes and should be the primary goal of ASCT incorporating the novel agents.

0511
BORTEZOMIB-BASED INDUCTION AND MAINTENANCE THERAPY IMPROVES OUTCOME IN MYELOMA PATIENTS WITH DELETION 17P - A SUBGROUP ANALYSIS OF THE HOVON65/GMMG-HD4 TRIAL

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Background. Chromosomal aberrations are important prognostic parameters in multiple myeloma (MM). By using interphase fluorescent in situ hybridization (FISH) on CD138-enriched plasma cells, specific changes in interphase cells can be detected, overcoming the lack of dividing cells required for conventional cytogenetics. Aims. We evaluated the association of FISH results and outcome. Methods. Pts were randomized to receive three cycles of VAD (arm A; vincristine, adriamycin, dexamethasone) or FAD (arm B; bortezomib, adriamycin, dexamethasone). All pts received one or two cycles of high dose melphalan (200 mg/m2) with autologous stem cell transplantation followed by maintenance therapy with thalidomide 50 mg daily (arm A) or bortezomib 1.3 mg/m2 once every 2 weeks (arm B), respectively, for a maximum of 2 years. Sites in Germany, the Netherlands and Belgium participated in this trial (n=833 pts). For the German pts (GMMG, n=399) FISH was performed in a single laboratory prior to start of treatment. Cytospecks of CD138 purified plasma cells were subjected to FISH with two-color probe sets for the detection of numerical changes for the following chromosome regions: 1q21/8p21, 6q21/15q22, 9q34/22q11, 11q23/13q14, and 17p15/19q13, as well as for the translocations t(4;14), t(11;14), and t(14;16). Results. For this analysis, FISH results from 354 (89%) of all GMMG pts were available (n=182 in arm A; n=172 in arm B). For all pts the median follow-up time from randomization was 38.9 months (mo). The most pronounced impact on prognosis was seen for t(4;14), del13q14, del17p15, and gain1q21, each significantly associated with poor prognosis with respect to progression-free survival (PFS) and overall survival (OS). However, deletion of chromosome 13q as exclusive chromosomal aberration without the presence of del(17p) and t(4;14) indicates no impact on outcome. A multivariable Cox PH Model identified t(4;14), del17p15, gain1q21, and ISS III as independent factors for PFS and OS. When comparing pts in the two arms for PFS and OS, we found that bortezomib-based treatment improves significantly the outcome in myeloma patients with del(17p) (3yr-PFS rates: A: 16%; B: 27%; p=0.030; 3yr-OS rates: A: 17%; B: 69%; p=0.014). Patients with t(4;14) or gain1q21 showed also a favourable outcome in arm B, although this difference was not of statistical significance. A multivariable Cox PH Model showed that patients with newly-diagnosed MM. All patients with high-risk chromosomal aberrations benefit at least in part from bortezomib-based treatment. The negative impact of del17p15 on PFS and OS could be significantly improved by the bortezomib-based treatment, suggesting that long-term administration of bortezomib should be considered as a standard of care for these patients.
0512 PROGNOSTIC RELEVANCE OF 18F-FDG PET/CT NEGATIVITY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS

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Background. 18F-FDG PET/CT has been reported to be a careful technique for a widespread screening of myelomatous lesions at the onset of MM. Moreover, FDG-PET has been identified as a valuable methods to carefully monitor response and predict clinical outcomes in various tumors, particularly in lymphoma. The incorporation of novel agents into ASCT allowed the achievement of unprecedented high rates of complete response (CR) in young multiple myeloma (MM) patients. Availability of new techniques to identify minimal residual disease (MRD), such as multiparametric flow cytometry or molecular biology, led to the demonstration of a correlation between the depth of response and prognosis. Aims. Aim of the present study was to prospectively analyze the prognostic relevance of PET/CT negativity after induction and ASCT in 192 newly diagnosed MM patients. Methods. By study design, all patients were studied with 18F-FDG PET/CT at baseline, after induction treatment, after ASCT, once/year during post-ASCT follow-up and at the time of relapse. Tumor number was described as negative, partial or focal. The number of focal lesions (FL), as well as size and associated standardized uptake values (SUV) were recorded. Extramedullary disease (EMD), if present, was described by location, size, number and SUV. Results. Twenty four percent of the patients had a negative PET/CT scan at diagnosis. Among PET/CT-positive patients, 44% showed ≥ 3 focal lesions (FLs), 46% had SUV values > 4.2 and in 6% EMD could be detected. These 3 variables adversely affected 4-year estimates of PFS and OS. Thirty seven percent of the patients were negative after induction, while PET/CT was negative in 65% after 3 months from ASCT. Persistence of severe FDG uptake (SUVmax ≥ 4.2) after induction predicted for shorter PFS at 4 years (P = 0.004). Complete FDG suppression post-ASCT conferred superior PFS and OS in comparison with persistence of FDG uptake. In particular, 4-year estimates of PFS and OS for negative patients were 66% and 89%, respectively, as compared to 45% and 65% for positive patients (P = 0.02 both for PFS and OS). In multivariate analysis, both severe PET/CT involvement at diagnosis (SUV > 4.2 and/or EMD) and persistence of FDG uptake after ASCT were independent predictors of worst PFS (SUV > 4.2: HR= 2.0, 95%CI: 1.13-3.72; EMD= HR= 15.0, 4.0.55.8; FDG uptake after ASCT= HR= 2.12, 1.19-3.77) and OS (EMD= HR= 6.99, 2.28-21.46; FDG uptake after ASCT= HR= 3.57, 1.03-12.39). Conclusions. These results provide demonstration that PET/CT at diagnosis and after treatment is a reliable tool for predicting prognosis in autografted MM patients and to identify patients at different risk of progression. In particular, post-ASCT complete FDG suppression is associated with extended PFS and OS. Based on these data, aims to evaluate MRD should include also imaging techniques such as PET/CT.

0513 EFFECTS OF ZOLERODRINACID (ZOL) VERSUS CLODRONATE (CLO) ON MYELOMA-RELATED ORGAN OR TISSUE IMPAIRMENT (ROTI) IN PATIENTS WITH MULTIPLE MYELOMA (MM) IN THE MRC MYELOMA IX STUDY

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Background. A key feature of MM is related organ or tissue impairment (ROTI), manifest as CRAB: hypercalcemia, renal impairment, anemia, bone lesions and infection. In the Medical Research Council (MRC) Myeloma IX study, ZOL significantly improved overall and progression-free survival versus CLO in patients undergoing initial therapy for MM (hazard ration [HR] = 0.84, P = 0.0118 for survival; HR = 0.86, P = 0.0179 for progression-free survival; Morgan et al. Lancet, 2010), but the effects on CRAB have not been reported. Aims. These exploratory analyses investigated whether ZOL provided benefits beyond CLO for CRAB in the Myeloma IX study. Both agents are indicated for the prevention of skeletal complications from MM in the United Kingdom; prevention of CRAB is, therefore, off label. Methods. Patients newly diagnosed with MM were assigned either to intensive or non-intensive treatment pathways and randomized to CVAD vs CTD or to MP vs CTDa, respectively. Within each treatment arm, patients were randomized to intravenous ZOL (4 mg q3-4wk, adjusted based on renal function) or oral CLO (1,600 mg/d), each of which was continued at least until disease progression. Safety was assessed by continuous adverse event monitoring and standard laboratory and imaging evaluations. All patients provided informed written consent. Results. Among the intent-to-treat population (N = 1,960), at a median follow-up 3.7 years, a total of 104 new osteolytic lesions were reported in 95 (9.7%) CLO-treated patients, compared with only 53 new osteolytic lesions in 46 (4.7%) ZOL-treated patients (P < 0.001). Overall, more patients treated with ZOL versus CLO had complete or very-good partial responses (P = 0.018 for the non-intensive pathway). During the first 3 months on study, 16 (1.8%) CLO-treated but no ZOL-treated patients died of renal failure (P = 0.025). In each group, 28 (2.9%) patients had hypercalcemia, respectively; as-compared to 45% and 65% for positive patients (P = 0.02 both for PFS and OS). In multivariate analysis, both severe PET/CT involvement at diagnosis (SUV > 4.2 and/or EMD) and persistence of FDG uptake after ASCT were independent predictors of worst PFS (SUV > 4.2: HR= 2.0, 95%CI: 1.13-3.72; EMD= HR= 15.0, 4.0.55.8; FDG uptake after ASCT= HR= 2.12, 1.19-3.77) and OS (EMD= HR= 6.99, 2.28-21.46; FDG uptake after ASCT= HR= 3.57, 1.03-12.39). Conclusions. These results provide demonstration that PET/CT at diagnosis and after treatment is a reliable tool for predicting progression in autografted MM patients and to identify patients at different risk of progression. In particular, post-ASCT complete FDG suppression is associated with extended PFS and OS. Based on these data, aims to evaluate MRD should include also imaging techniques such as PET/CT.
LESTAURTINIB INHIBITION OF THE JAK/STAT SIGNALING PATHWAY IN CLASSIC HODGKIN LYMPHOMA (CHL) CELLS AND IN PATIENTS LYMPH NODES INHIBITS PROLIFERATION AND INDUCES APOPTOSIS

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Background. Standard cytotoxic chemotherapy for CHL has changed little in 30 years; the treatment for patients with relapsed or refractory disease remains challenging and novel agents are under development. JAK/STAT constitutive activation plays an important role in the pathogenesis of CHL. Lestaurtinib is an orally bioavailable multikinase inhibitor that has recently been shown to inhibit JAK2 in myeloproliferative neoplasms. Its potential role in CHL therapy is unknown. Aims. Firstly, to analyze the in vitro effectiveness of Lestaurtinib in five CHL cell lines and its role in the JAK2/STAT5 signaling pathway. Secondly, to analyze for the first time the effect of Lestaurtinib in lymph nodes from CHL patients by flow cytometry. Methods. Five CHL cell lines, L-428, L-1236, L-540, HDLM-2 and HD3-MY-Z were assayed for proliferation and apoptosis after 48h of treatment with Lestaurtinib or DMSO (control). Cell growth was determined using CellTiter 96 AQueous One Solution Cell Proliferation Assay and apoptosis was measured using CaspaseGlo 3/7 kit (Promega). After 1 hour of incubation with Lestaurtinib, the levels of the JAK2 pathway proteins, JAK2, phospho-JAK2 (Tyr1007/1008), STAT5, phospho-STAT5 (Tyr694), STAT3 and phospho-STAT3 (Tyr705), were analyzed by Western Blot. BCL-XL mRNA levels were quantified using TaqMan Gene Expression assays (Applied Biosystems). CHL patients were diagnosed at the Haematology Department from the University Hospital del Mar, Barcelona. Median age was 29 (range 24-43); 3 were nodular sclerosis and one lymphocyte-rich subtype. All patients were EBV- and 2 were stage IIA and 2 stage IIIA. By flow cytometry, we evaluated 750,000 cells from lymph nodes cultured for 24 hours with 300nM of Lestaurtinib or DMSO. Hodgkin Reed-Sternberg (HRS) cells were gated by the expression of CD40-PE-Cy5, CD30-Pacific Blue and CD50-PE, and the absence of CD3-APC-Cy7 (BD Bioscience). Viability was analyzed using FITC AnnexinV (BD Bioscience). Samples were analyzed on a FACSCanto II (Becton Dickinson). Results. At 48h, a dose-dependent cell growth inhibition (23-66% at 300nM) and apoptotic increment (10-64% at 300nM) were observed in all cell lines. Moreover, Lestaurtinib inhibited JAK2, STAT5 and STAT3 phosphorylation and reduced the mRNA expression of its downstream antiapoptotic target Bcl-xL. Additionally, we have analyzed the effect of Lestaurtinib in lymph nodes from four CHL patients. We have evaluated the effect of treatment with 300nM of Lestaurtinib in the subpopulation of lymph node cells CD30+, CD40+, CD95+ and CD5-, which contain HRS cells. After 24h, cell viability had decreased in three of the four cases by 22%, 35% and 24% versus control cells. In the non-respondent patient, we increased the treatment dose to 1µM and then we observed a reduction in cell viability by 12%. In order to shed light on the potential toxicity of Lestaurtinib, we have also analyzed cell viability in lymph node CD3+ cells after treatment with 300nM of Lestaurtinib and observed no decrease of viability (mean versus control=100.5%; range: 90%-119%). Summary/Conclusions. Our findings provide, for the first time, a molecular rationale for testing JAK2 inhibitors, specifically Lestaurtinib, in HL patients.

Figure 1.
LONG TERM FOLLOW-UP OF PATIENTS TREATED WITH INVOLVED-FIELD COMPARED WITH EXTENDED-FIELD RADIOTHERAPY AFTER CHEMOTHERAPY FOR HODGKIN’S LYMPHOMA: 10 YEAR-ANALYSIS OF THE HD8 TRIAL OF THE GHSG

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Background/Aims. In the HD8 trial of the German Hodgkin Study Group (GHSG), we investigated whether radiotherapy (RT) can be reduced without loss of efficacy from extended field (EF) to involved field (IF) after four cycles of chemotherapy. The current 10 year follow-up analysis was conducted to assess the influence of the two modalities with regard to long-term outcome rates and toxicities including secondary malignancies. Methods. Between 1998 and 1998, patients with newly diagnosed early-stage unfavorable HL were randomized to receive four cycles of chemotherapy followed by either RT of 30 Gy EF + 10 Gy to bulky disease (arm A) or 30 Gy IF + 10 Gy to bulky disease (arm B). Of 1,204 patients randomly assigned to treatment, 1064 patients were informative and eligible for the arm comparison (532 patients in each treatment arm; drop-outs before RT excluded). Results. Patient demographics and clinical characteristics were well balanced between the two treatment arms. The median observation time was 114 months. Survival rates at 10 years after start of radiotherapy revealed no differences for arms A and B with respect to freedom from treatment failure (FFTF; 79.8% and 79.7%), progression free survival (PFS; 79.5% and 80.0%) and overall survival (OS; 86.4% and 87.3%). Non-inferiority of the experimental arm (IF-RT) was proven with statistical significance for the primary endpoint FFTF (95%CI for HR 0.72-1.25). However, patients 60 years or older had an inferior outcome when treated with EF-RT compared with IF-RT. Acute toxicity from radiotherapy including nausea, leucopenia, thrombocytopenia, gastrointestinal and pharyngeal toxicity were more frequent in the IF arm. A total of 15% of patients in arm A and 12.2% of patients in arm B died; causes of deaths were mainly secondary malignancies (5.3% vs. 3.4%), HL (3.2% vs. 3.4%), and cardiovascular disease (1.7% vs. 2.1%). Interestingly, there were more secondary malignancies (n=58 vs. n=45), especially AMLs (n=11 vs. n=4) after combined modality treatment including EF-RT than IF-RT. However, longer follow-up is needed for statistical significance and evaluation of other long-term side effects. Summary/Conclusions. Radiotherapy volume size reduction from EF to IF after chemotherapy does not result in inferior long-term outcome and produces less acute and long-term toxicities in patients with early-stage unfavorable HL.

TARC BIOMARKER TRENDS OBSERVED IN A PIVOTAL PHASE II STUDY OF ORAL PANOBINOSTAT IN PATIENTS WITH RELAPSED/REFRACTORY HODGKIN LYMPHOMA FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANT

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Background. Panobinostat is an oral pan-deacetylase inhibitor (pan-DACi). TARC, thymus and activation-regulated chemokine (TARC) is produced by Reed-Sternberg cells and is a potent growth factor for Reed-Sternberg cells and is a potential biomarker to monitor disease control. The level of TARC reduction at cycle 1 day 15 was highest in patients achieving CR and PR, providing further evidence to support the mechanism of action of DACi in Hodgkin lymphoma. Further analysis is ongoing to correlate changes in TARC vs time to response and target lesion reduction, and these updated results may support TARC as a potential biomarker for monitoring Hodgkin lymphoma response to panobinostat.

OBJECTIVE RESPONSES WITH BRENTUXIMAB VEDOTIN (SGN-35) IN PATIENTS WITH RELAPSED OR REFRACTORY HODGKIN LYMPHOMA (HL) WHO REFUSED OR WERE INELIGIBLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT)

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Background. Brentuximab vedotin (SGN-35) is an anti-CD30 antibody conjugated to the highly potent antimitotubule agent, monomethyl auristatin E (MMAE), by a plasma-stable linker. Brentuximab vedotin binds to CD30 on the cell surface, internalizes, and releases MMAE inside the cell via lysosomal degradation. Binding of MMAE to tubulin disrupts the microtubule network, induces cell cycle arrest, and results in apoptotic death of the CD30-expressing tumor cell. Brentuximab vedotin induced durable objective responses in association with manage-
able adverse events in patients with relapsed or refractory HL after ASCT (Chen et al., ASH 2010). Aims. To characterize the safety and efficacy of brentuximab vedotin in a population of patients with relapsed or refractory HL who refused or were ineligible for ASCT (“pre-ASCT” patients). Methods. Pre-ASCT patients were enrolled per study entry criteria in 2 phase 1 multicenter studies. In Study SG035-0001, patients received brentuximab vedotin IV q3 weeks at: 0.1, 0.2, 0.6, and 1.2 mg/kg (1 patient each); 1.8 mg/kg (5 patients); and 2.7 mg/kg (4 patients). In Study SG035-0002, patients received brentuximab vedotin IV q1 week, 3 out of 4 weeks, at: 0.4 mg/kg (2 patients), 0.8 mg/kg (1 patient), 1.0 mg/kg (8 patients), 1.2 mg/kg (1 patient), and 1.4 mg/kg (3 patients). Informed consent was obtained for all patients. Results. Twenty pre-ASCT patients were enrolled across the 2 studies. The median age was 31.5 years (range, 12-87), 65% were male, baseline ECOG performance status was 0 (60%), 1 (30%) or 2 (20%). The median number of prior systemic chemotherapy regimens was 3 (range, 1-7), and 45% of patients had received prior radiotherapy. Relative to the post-ASCT population, a higher proportion of pre-ASCT patients had primary refractory disease and were refractory to their most recent prior therapy. The incidence of bone marrow involvement and baseline B symptoms was also higher in the pre-ASCT population. Adverse events (AEs) of any grade in ≥25% of patients were fatigue, nausea, pyrexia, diarrhea, vomiting, back pain, decreased appetite, anemia, night sweats, and weight decreased. Eleven of 20 patients (55%) experienced AEs with a maximum severity of Grade 3 and treatment-related serious AEs were reported in 3 patients (15%). No deaths occurred within 30 days of the last dose of brentuximab vedotin. Objective responses (Cheson 2007) were observed in 6 of the 20 pre-ASCT patients (30%); 2 CR and 4 PR. Median duration of objective response could not be estimated because only 1 of the 6 patients had disease progression or death by the time of study closure. Censored response duration in the remaining 5 responders ranged from 29.6+ to 60.1+ weeks. Summary/Conclusions. Brentuximab vedotin was associated with manageable adverse events in a population of patients with relapsed or refractory HL who refused or were ineligible for ASCT, with a safety profile comparable to that observed in post-ASCT patients. The demonstration of objective responses suggests that anti-tumor activity is not limited to patients who received brentuximab vedotin after ASCT and warrants further studies in earlier lines of therapy.

0519
HIV-RELATED HODGKIN’S LYMPHOMA (HIV-HL): RESULTS OF PROSPECTIVE MULTICENTER TRIAL

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Background. The outcome of patients (pts) with HIV-HL has improved since the introduction of highly active antiretroviral therapy (HAART). However, standard therapy for HIV-HL has not been defined. Aims. The current trial was initiated to investigate a risk adapted treatment strategy in pts with HIV-HL as established in HIV-negative pts with HL. Methods. Pts. were planned to receive 2x ABVD + 30 Gy involved field (IF) radiation for early stage (ES) favourable HL (stage I/II without risk factors), 4x BEACOPP baseline + 30 Gy IF for ES unfavourable HL (extranodal involvement, large mediastinal mass, ≥ 3 lymph node areas involved), and 6-8 x BEACOPP baseline for advanced stage HL. BEACOPP should be replaced by ABVD in pts with far advanced HIV-infection. HAART was given concomitantly with chemotherapy. The primary endpoint was tolerability and treatment related mortality. Secondary endpoints include event free survival (EFS) and overall survival (OS). Results. From 03/2004 to 12/2010 105 pts (8 females) were included in the trial. 28/105 pts (22%) had ES favourable HL, 14 (13%) ES unfavourable HL, and 68 (65%) advanced stage HL. B-symptoms were present in 69 pts (66%) and the mixed cellularity subtype was found in 63 pts (60%). 28 pts (27%) had a prior AIDS defining illness. The median CD4 count at HL diagnosis was 223/µl (range 7-967) and 57 pts (54%) had an HIV-viral load below the detection limit. The median time from HIV diagnosis to HL diagnosis was 5.8 yrs (range 0 - 26). In advanced stage HL grade 3/4 toxicity occurred in 13/13 pts under ABVD and 45/60 pts (75%) under BEACOPP with non haematological toxicity being more frequently observed under BEACOPP than under ABVD (46% vs. 39%). 5 pts died of neutropenic sepsis after the 1st, 7th (n=2) and 8th cycle of BEACOPP, and after 1 cycle of ABVD, respectively. So far response data are available from 101 pts. After a median follow-up of 23.1 months 20/21 pts (95%) achieved a CR/CRu. In pts with advanced HL the CR/CRu rate was 87% (58/67). Of 6 pts with relapsed/refractory HL 2 received an autologous stem cell transplant resulting in a 2nd remission and 4 pts died of progressive disease. 10 of 105 pts (9.5%) have died. Apart from neutropenic sepsis causes of death were progressive HL (n=4) and progressive HIV-infection (PML, n=1). The 2-year OS of the entire study population is 90.6% without significant differences between early, intermediate and advanced stage HL. However, pts with both, advanced HL and advanced HIV-infection had a significantly worse OS (p=0.025).

Conclusions. In pts with HIV-HL risk-adapted CT and concomitant HAART is feasible and effective. However, pts must closely be monitored for neutropenic infections. These data suggest that the prognosis of HIV-HD may approach results achieved in the HIV-negative population with HL.
Chronic myeloid leukemia - Biology

0520
THE TUMOR SUPPRESSOR PP2A AS A THERAPEUTIC TARGET FOR ERADICATION OF TKR-RESISTANT PH+ LEUKEMIC STEM CELLS

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Background/aims: The success of tyrosine kinase inhibitors (TKIs) depends on the addiction of Philadelphia-positive (Ph+) CML progenitors to BCR-ABL1 kinase activity. However, CML quiescent hematopoietic stem cells (HSC) are TKI-resistant and represent an active disease reservoir. We hypothesize that this innate drug-resistance depends on inhibition of the tumor suppressor protein phosphatase 2A (PP2A). PP2A can be reactivated by FTY720, a drug that targets CML but not normal progenitors. Here we investigated the mechanism controlling survival/self-renewal of quiescent leukemic HSCs and their sensitivity to PP2A-activating drugs. Methods: HSCs from CML (n=68) and healthy (n=12) donors were FACs-isolated, and the biologic importance of TKIs.

0521
COMBINATION OF THE HEDGEHOG PATHWAY INHIBITOR LDE225 AND NILOTINIB TARGETS THE LEUKEMIC STEM CELL POPULATION IN CHRONIC MYELOID LEUKAEMIA

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Background. Although effective in inducing cytogenetic and molecular remission in chronic myeloid leukaemia (CML), tyrosine kinase inhibitors do not eliminate leukaemia stem cells (LSC), potentially resulting in relapse/progression. Therefore alternative strategies are required for eradication of CML LSC. The Hedgehog (Hh) pathway, a developmental pathway regulating stem cell fate, is active in BCR-ABL+ LSC and contributes to CML LSC maintenance via up-regulation of Smoothened (SMO). Thus inhibition of SMO may specifically target CML LSC. Aim.

To assess the effect of LDE225 – a clinical grade SMO inhibitor (Novartis) - alone and in combination with nilotinib in chronic phase (CP)-CML. Methods. Baseline global gene expression was assessed in primed CD34+ normal and CP-CML cells using the HuGene 1.0 ST array and Taqman® qRT-PCR. CD34+ primary CP-CML cells were cultured in the presence of LDE225-nilotinib prior to analysis, inoculation into colony forming cell assays (CFC) or long-term culture initiating cell assays (LTC-IC) or transduction into NOD-SCID γ-chain (NSG) mice. The Scl-tαa-BCR-ABL1 murine model of CP-CML was used to assess effects of in vivo treatment with LDE225 (80μg/kg/day) ± nilotinib (50μg/kg/day) or vehicle over 21 days (Koschmieder et al; Blood 2005;105:244-54). Results. We detected differential expression of key Hh elements and targets between normal and primed CD34+ normal and CP-CML cells. Furthermore, Hh targets (Gli1 and Ptc2) in CD34+ CP-CML cells were inhibited following 72 hours exposure to LDE225. Increasing concentrations of LDE225 (1nM-1μM) had no effect on viability, proliferation or cell cycle status on primary CD34+ CP-CML cells compared to untreated controls. LDE225 did not affect CFC readout after 14 days, however, we noted a significant reduction in secondary colony formation following re-plating after exposure to LDE225 (50nM LDE225; 62%-p<0.02%) alone or combined with nilotinib (LDE225 10nM & nilotinib 5μM; 73%-p<0.03). LDE225 also reduced the LTC-IC recovery of CD34+ CP-CML cells compared to untreated controls (40%-p<0.03) and combination treatment (LDE225 10nM & nilotinib 5μM) resulted in significantly reduced LTC-IC frequency versus nilotinib alone (85%-p<0.007). CD34+ CP-CML cells, exposed to 10nM LDE225, 5μM nilotinib or combination for 72 hours were transplanted into NSG mice. Combination treatment reduced engraftment of CML CD45-, CD34+/45+ and CFC comparison with control (p<0.02 / p<0.008 respectively). FISH demonstrated a 1.3-fold and 1.8-fold reduction in engrafted leukemic cells with nilotinib and LDE225 respectively but a 6-fold reduction following combination treatment. LDE225&nilotinib did not affect engraftment of normal CD34+ cells. In the Scl-tαa-BCR-ABL1 murine model of CML LDE225-nilotinib reduced the number of splenic Lin’Sca-1’KitFlt3-1CD34+1CD150-CD45- (LT-HSC cells) (p<0.01) compared to control, but did not affect bone marrow (BM) LT-HSC. Nilotinib did not reduce LT-HSC numbers in spleen or BM. Mice treated with combination therapy demonstrated enhanced post-treatment survival compared with other experimental arms. Secondary transplantation experiments indicated that recipients of BM or spleen cells from combination treated mice had a reduced incidence of leukaemogenesis. Conclusion. LDE225 targets CP-CML LSC in vitro and in a murine model of CML. LDE225 combined with nilotinib represents a promising novel strategy for eradicating the LSC population in CP-CML patients.

0522
ENHANCED EXPRESSION OF THE HEDGEHOG PATHWAY TARGET GENE PTC1 INHIBITS PREDICTOR RESPONSE TO IMATINIB IN CHRONIC MYELOID LEUKAEMIA

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Background. Tyrosine kinase inhibitors (TKI) for chronic myeloid leukemia have yielded great success. However, BCR-ABL1-positive leukemic stem cells (LSCs) can persist despite lifelong TKI therapy and are believed to be a source of disease relapse and eventual progression. Recent studies indicate that activity of the Hedgehog (Hh) signaling pathway is crucial for survival of LSCs in CML. The level of Hh activity can be quantified via expression of its downstream target genes GLI1 and PTC1. Aim. The aim of this study was to investigate the range of expression of PTC1 in CML patients at diagnosis and to compare expression of Hh activity, to clind the level of Hh activity with clinical outcome and PTCH1. Methods. Real-time quantitative PCR was used to measure PTC1 expression in relation to a housekeeping gene (GUSB). RQ-PCR was performed on cDNA samples from peripheral blood granulocytes taken from 85 unselected CML patients at the time of diagnosis. Imatinib was given as a first line therapy for patients in chronic phase. A second cohort of 31 CML patients was identified for validation of clinical associations. Probabilities of OS, PFS and EFS were calculated using the Kaplan-Meier method. A receiver operating characteristic (ROC) curve method was used to calculate a threshold for poor response. Results. The median level of PTC1 expression was greater than that of GUSB was 3x10-4 (range 0 to 3.76x10-1). Importantly, the 59 patients with low expression of PTC1 at diagnosis (calculated as on or below the 95th percentile of the expression range) had a superior seven year probability of OS (0.85) compared with patients (p<0.005 respectively). Importantly, the 59 patients with low expression of PTC1 at diagnosis (calculated as on or below the 95th percentile of the expression range) had a superior seven year probability of OS (0.85) compared with patients (p<0.005 respectively).
CANCEROUS INHIBITOR OF PP2A (CIP2A) INHIBITS PP2A AND STABILISES PIM1 AND C-MYC IN CML LEADING TO BLAST CRISIS

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Background. BCR-ABL1 tyrosine kinase activity induces and maintains chronic myeloid leukaemia (CML), but the molecular factors that contribute to disease progression are not well understood. PP2A is a phosphatase which regulates cell proliferation, differentiation and survival. Dysregulation of PP2A in CML and as a consequence of CML stem cell phenotype is a mechanism by which CIP2A predisposes patients to disease progression. In MNC and CD34+ cells taken at diagnosis from patients destined to progress to blast crisis (BC), we have shown that CIP2A functionally inhibits PP2A, and that levels of CIP2A detected in diagnostic samples can prospectively predict disease progression. We have previously shown that CIP2A is not suppressed by imatinib treatment and 2) CIP2A levels rise as patients progress into BC, further supporting PP2A function. As CIP2A levels rise BCR-ABL1 tyrosine kinase activity increases. Aim. The aim of this study was to investigate the mechanism by which CIP2A predisposes patients to disease progression.

Methods. CIP2A, PP2A, pY307-PP2A and PIM1 proteins were assessed by flow cytometry in 31 newly diagnosed chronic phase patients. CIP2A, PP2A, PIM1 and c-Myc were assessed by ELISA. CIP2A siRNA was transfected into K562 cells. Results were analysed in patients stratified according to their eventual clinical outcome - cytogenetic response; namely 94.9% vs 65.4% (p=0.007), 72.0% vs 23.7% (p=0.0007), 84.1% vs 58.6% (p=0.0001), 98.1% vs 53.2% (p<0.0001) and 97.4% vs 58.9% (p<0.0001). The expression of CIP2A considered as a continuous variable was also predictive for the achievement of CCyR (RR=0.68, p=0.01), MMR 0.49, p=0.004), EFS (RR=0.56, p=0.0001), PFS (RR=0.67, p=0.001) and OS (RR=0.76, p=0.006). Expression of CIP2A was an independent predictor for OS, EFS and CCyR and its predictive value was independent of Sokal score. Summary. Our data demonstrate significantly higher pS62-Myc and c-Myc levels than in those patients who do not progress (p=0.04, p=0.002 respectively) suggesting high c-Myc levels indicate a high risk of disease progression. Phosphorylation of c-Myc on serine 62 stabilises c-Myc from degradation; PIM1 is also a target for PP2A. The proportion of Ph+ cells was 14% and 56% in CD34+CD38- and CD34+CD38+ fractions respectively. Patients who achieved MMR at 6 months time-point had significantly lower Ph+ cells at diagnosis compared to patients without MMR (n=21) at 6 months (94.9% vs 65.4%, p<0.001), enlarged spleen (r=0.43, p=0.0055), high blood blast percentage (r=0.57, p=0.0001) and low hemoglobin concentration (r=0.47, p=0.002) relative to normal counterparts. Variability in Hh pathway activity in CML HSCs therefore represents a plausible mechanism by which the CML clone in some patients may escape the inhibitory effects of imatinib to become a source of frank relapse.

0523

THE PROPORTION OF LEUKEMIC STEM CELLS (PH+ CD34+CD38-) IN BONE MARROW OF NEWLY DIAGNOSED PATIENTS WITH CML CORRELATES WITH EARLY CYTOGENETIC AND MOLECULAR RESPONSE TO IMATINIB OR OTH OR THERAPIES

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Background. In vitro studies have suggested that CML stem cells are resistant to bcr-abl inhibitors. However, no prospective clinical studies have evaluated their effect on leukemic stem cells in patients. In addition, the prognostic value of leukemic stem cell burden at diagnosis is unknown. Aims. To analyze the proportion of Ph+ putative leukemia stem cells in CML patients at diagnosis and during imatinib and dasatinib treatment (TRI therapy), and correlate the leukemic stem cell burden with disease eradication kinetics and early treatment response. Patients and Methods. 42 newly diagnosed chronic phase CML patients within the Nordic countries were enrolled in a Phase II study (NordCML006) comparing the effect of dasatinib 100 mg (n=21) to imatinib 400 mg (n=21) at the stem cell level. Ph+ candidate leukemic stem cells were analyzed at 0, 1, 3, and 6 months after start of therapy. After pre-selection of CD34+ cells from BM aspirates, the cells were fractionated into CD38+ positive (CD34+CD38+) and negative (CD34+CD38-) pools with FACS. The proportion of Ph+ cells in different fractions was determined by interphase FISH for BCR-ABL1. Results. Measurement of Ph+ stem cells was feasible in most patients at all time-points. The median percentage of Ph+ cells at diagnosis was significantly lower in CD34+CD38- fraction when compared to CD34+CD38+ fraction or to unFractionated BM (79%, range 0.6-100%; 96%, 50-100%; p=0.004 respectively). The proportion of Ph+ cells in CD34+CD38+ fraction at diagnosis correlated with high leukocyte count (r=0.50, p<0.001), enlarged spleen (r=0.43, p=0.0055), high blood blast percentage (r=0.57, p=0.0001) and low hemoglobin concentration (r=0.47, p=0.002). During TKI therapy, the proportion of Ph+ cells decreased rapidly in both stem cell fractions. At 1 month, the median proportion of Ph+ cells was 14% and 56% in CD34+CD38- and CD34+CD38+ fractions, respectively compared to 71% in whole BM (p=0.0002, n=36). At 3 month, the respective numbers were 0.18%, 0.19% and 0.80% and (p=0.03, n=27). The proportion of Ph+ cells in the CD34+CD38- fraction at diagnosis correlated with high leukocyte count (r=0.50, p<0.001), enlarged spleen (r=0.43, p=0.0055), high blood blast percentage (r=0.57, p<0.0001) and low hemoglobin concentration (r=0.47, p=0.002). Pre-treatment leukemic stem cell burden also correlated with cytogenetic response during TKI therapy at 1, 3 and 6 months time-points (r=0.67, p<0.0001; r=0.52, p=0.0017; r=0.43, p=0.015, respectively). Patients who achieved MMR at 6 months time-point (n=17) had significantly lower proportion of Ph+ CD34+CD38- cells at diagnosis compared to patients without MMR (n=21) at 6 months (70% vs 6%, p=0.05). Grade 2 hematological adverse effects were more common during first 3 months of therapy in patients with high leukemic stem cell burden at diagnosis (55% vs 19%, p=0.02). Conclusions. Our results indicate that the proportion of Ph+ stem cells at diagnosis is a key biological marker and correlates with cytogenetic and molecular response as well as hematological toxicity within first 6 months of treatment. Successful TKI therapy rapidly eliminates Ph+ cells from the stem cell compartments in vivo. The effect of therapy by the treatment arm will be analyzed when all patients have reached the primary endpoint at 6 months time-point.
Platelets and bleeding disorders

0525
A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED PHASE II TRIAL ON THE EFFICACY, SAFETY AND TOLERABILITY OF E5501 (AKR501) IN SUBJECTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (cITP)

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Background. Thrombopoietin (TPO) receptor agonists mimic TPO, the endogenous platelet production regulator, and have demonstrated efficacy in randomized controlled trials in chronic immune thrombocytopenia (cITP) patients who relapsed after first- and/or second-line agents. Aims. E5501 (previously AKR501) is a novel, orally-active, once-a-day TPO agonist which increased platelet counts in healthy volunteers. This study investigated the efficacy and safety of E5501 in subjects with cITP. Methods. This was a Phase II, multicenter, randomized, double-blind, placebo-controlled, dose-ranging, parallel group 4-week study (501-CL-003). Subjects had cITP that was refractory to, or had relapsed after, at least one prior therapy, and had a baseline platelet count of <80 x 10^9/L, or <50 x 10^9/L if they were on stable corticosteroid therapy. Sixty-four subjects were enrolled and randomized in the ratio 3:3:3:3:1 respectively to E5501 (2.5, 5, 10 or 20 mg) or placebo, administered orally, once daily for 28 days. Response was assessed by weekly platelet count and the primary endpoint was responder rate at Day 28, with responders defined as having a platelet count ≥50 x 10^9/L that had risen by ≥20 x 10^9/L above baseline. Results. A dose-dependent increase in responder rate at Day 28 was observed among subjects receiving E5501 (Table 1). Responder rate at Day 28 was significantly higher in the E5501 20 mg group (80.0%) than the placebo group (0%; p=0.0036) and the E5501 2.5 mg group (13.3%; p=0.0007). Median platelet counts at Day 28, and change above baseline, increased dose dependently in subjects receiving E5501 (Table 1). Median platelet counts at Day 28, and change above baseline, increased dose dependently in subjects receiving E5501 (Table 1). Median platelet counts at Day 28, and change above baseline, increased dose dependently in subjects receiving E5501 (Table 1). Table 1.

Table 2.

A dose-dependent increase in responder rate at Day 28 was observed among subjects receiving E5501. In the E5501 20 mg group, 93.3% of subjects achieved early response with E5501 20 mg, 76.9% maintained platelet response at Day 28, and change above baseline, increased dose dependently in the E5501 20 mg group (80.0%) than the placebo group (0%; p=0.0036) and the E5501 2.5 mg group (13.3%; p=0.0007). Median platelet counts at Day 28, and change above baseline, increased dose dependently in the E5501 20 mg group (80.0%) than the placebo group (0%; p=0.0036) and the E5501 2.5 mg group (13.3%; p=0.0007). Median platelet counts at Day 28, and change above baseline, increased dose dependently in the E5501 20 mg group (80.0%) than the placebo group (0%; p=0.0036) and the E5501 2.5 mg group (13.3%; p=0.0007).

Conclusion. E5501 was effective in increasing platelet counts in subjects with cITP at doses of 5, 10, and 20 mg daily, was well tolerated and had an acceptable safety profile. These data support continued development of E5501 as an effective treatment in patients with cITP.

0526
A FAMILY WITH TYPE 2M VWD WITH NORMAL VWF:RCO BUT REDUCED VWF:CB DUE TO A M1761K MUTATION IN THE A3 DOMAIN

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Type 2 VWD is characterised by a qualitative defect in VWF and is diagnosed by demonstration of a discrepancy between circulating plasma levels of VWF and its functional activity. Type 2 VWD is subdivided into types 2A, 2B, 2M and 2N. Type 2A and 2M variants show decreased platelet binding but type 2A VWD is also associated with an absence of high molecular weight multimers. Type 2B variants have increased affinity for platelet glycoprotein 1b. Type 2N VWD refers to variants with a decreased affinity for F8. A 17-year-old presented with menorrhagia, Fe deficiency, epistaxis and bruising (bleeding score 5). Both her sisters and mother had menorrhagia and bruising, one sister had nose bleeds and the mother had a PPH (bleeding scores 2, 3 and 4). The principal detectable abnormality was defective collagen binding. Genetic analysis showed the mutation c.5282 T>A, p.M1761K in the A3 domain of VWF. All have the same mutation, normal RIPA and normal multimers. The table shows the results including VWF:CB measured by two different kits. Four previous mutations with this phenotype have been described S1731T, W1745C, S1783A, H1786D. Our novel mutation was picked up by the Corogenix assay (equine type III collagen) but not by the Technozym assay (pepsin-digested human type III collagen) which demonstrates that this sub-type of type 2M VWD will be missed if VWF:CB assays are not performed and that different assays may differ in their sensitivities. The 1994 definition of type 2M VWD depended on “decreased platelet-dependent function” which defined patients with VWF GPIb binding site defects who have reduced VWF:RCO. This family would have remained unclassified. In the recently updated ISTH SSC VWD classification isolated collagen binding defects are included within the 2M subgroup. Impairment of VWF-dependent platelet adhesion to the endothelium in type 2M VWD occurs in the presence of a full range of VWF multimers. This is a key characteristic of the 2M subtype. There are 3 important differences with the 2M group. 1. 2M mutations are clustered in the A1 domain (residues 1260-1471) of VWF, whereas these mutations af-
fecting collagen binding are found in A3. 2. M2 is characterised by impaired binding to platelet GpIbα, demonstrated by reduced ratio of RCo:Ag. 3. A clinically important feature of the M2 phenotype is that patients with M2 VWD have an increased risk of bleeding complications due to reduced fibrinogen binding.

**Table 1.**

<table>
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<tr>
<th>Subcutaneous Injections of Low-Dose Anti-CD20 Veltuzumab for Patients with Relapsed Immune Thrombocytopenia (ITP)</th>
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<tr>
<td>A Liebman, N Saleh, J Bussel, B Bernstein, G Negrea, C Onyegbula, M Farber, B Berndolf, A Horne, K Teob, A Wegen, D Goldenberg</td>
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<tr>
<td>Keck School of Medicine of University of Southern California, Los Angeles, CA, United States of America</td>
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<td>Georgia Cancer Specialists, Atlanta, GA, United States of America</td>
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<td>NY Presbyterian Hospital, New York, NY, United States of America</td>
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<td>Erie County Medical Center, Buffalo, NY, United States of America</td>
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<td>Low Country Cancer Care Associates, Savannah, GA, United States of America</td>
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<td>Center of Hope for Cancer and Blood Disorders, Rivardale, GA, United States of America</td>
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**Background.** Subcutaneous (SC) injections of veltuzumab, a 2nd-generation humanized anti-CD20 monoclonal antibody with structure-function differences from rituximab, may offer potential benefits to both patients and the healthcare system. Aims. A multicenter, phase II/I study to evaluate SC veltuzumab in adults with primary ITP who failed ≥1 standard therapy and presented with platelets ≤50K/μL, but without major bleeding. Methods. All patients received 2 doses of veltuzumab 2 weeks apart (without steroids), initially administered IV, but then by SC injection after a higher concentration formulation. All patients received 2 doses of veltuzumab 2 weeks apart (without steroids), initially administered IV, but then by SC injection after a higher concentration formulation became available. By recent international working group categories, efficacy was evaluated separately for patients with newly-diagnosed or persistent disease (≤1 year duration) compared to patients with chronic disease (>1 year), with best responses (on at least 2 occasions, one week apart) classified as complete (CR, >150K/μL), partial (PR, 50-150K/μL), or minor (MR, 30-50K/μL). Adverse events (AEs) and safety laboratories were evaluated by NCI CTC v3 toxicity grades. Other evaluations included circulating B-cell levels (CD19), veltuzumab serum levels, and human anti-veltuzumab antibody (HAHA) titers. Results. Of 36 patients now entered, 7 received IV veltuzumab doses of 80 μg/kg, but then switched to SC veltuzumab. The CRs were durable, with median relapse-free survival currently 1.2 years (0.3 - 1.9 yr) and 5/7 CRs still continuing. Most PRs and MRs relapsed before 6 months, and of 7 patients then retreated, most obtained responses comparable to their initial response. B cells were depleted rapidly with both IV and SC dosing with recovery starting 12 to 16 months after treatment. Compared to IV dosing, SC veltuzumab had slower release over several days with lower serum levels, but comparable availability/exposure. Four patients developed low-level HAHA titers of uncertain clinical significance.

Conclusions. Low-dose SC veltuzumab was convenient, well tolerated and with promising activity in relapsed ITP. With only 2 SC doses, patients with limited disease duration of ≤1 year achieved high rates of objective responses (88%), including 38% durable CRs. In patients with longer-standing disease, CRs occurred less frequently, but there was activity (61% CRs), and more extended dosing SC regimens may be required in this more refractory population.
INCIDENCE AND DETERMINANT OF BLEEDING IN DIFFERENT TYPES OF VON WILLEBRAND DISEASE: RESULTS OF A PROSPECTIVE MULTICENTER COHORT STUDY ON 797 ITALIAN PATIENTS

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Background. von Willebrand disease (VWD) is the most common inherited bleeding disorder and is due to quantitative and/or qualitative defects of von Willebrand factor (VWF). Despite the improved knowledge of this disorder, no data on the incidence and determinants of bleeding requiring specific treatments are available up to now. Aims and design of the study. To determine the incidence and determinants of bleeding requiring therapy with DDAVP and/or VWF/FVII concentrates in patients with VWD, a national registry (RENAWI) was organized to collect detailed retrospective information. Patients included in RENAWI were then followed up for one year and prospective data on number, type and management of bleeding episodes were analyzed. Methods. All patients were diagnosed following recommendations of the ISTH-SSC-SC on VWF, with bleeding score (BS) calculated at enrollment. Diagnosis of VWD was confirmed by the coordinating center using multimeric analysis in plasma and mutations of VWF gene. For different risk categories, the incidence of bleeding (mucosal and non-mucosal) was calculated. Bleeding-free survival was computed with the Kaplan-Meier method, and a Cox’s proportional hazard model was used to calculate the risk of bleeding [expressed as hazard ratio (HR)]. Results. Further highlight the value of high quality registry data for the investigation of rare diseases.

0529

IMPROVING OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The application of the combination of rituximab with a fludarabine-containing chemotherapy regimen in recent years has significantly improved the results of overall and disease-free survival of patients with chronic lymphocytic leukemia (CLL). Aims. To study the efficiency of maintenance with rituximab after induction chemotherapy or immuno-chemotherapy in CLL patients. Materials and Methods. The study included 213 patients in remission. The age of patients ranged from 54 to 76 years (median, 59 years). Remission was induced after a RFC program in 117 patients and FC in 96 patients. Complete remission (CR) was observed in 121 (57%) patients, and partial remission (PR) in 92 (43%). The patients were randomly assigned to either observation (158 patients) or supporting rituximab therapy in the form of 4 weekly injections (375 mg/m2) every 6 months over 2 years (60 patients). Results. The patients treated with a RFC regimen with subsequent rituximab maintenance had a significantly lower frequency of relapse and death compared to the observation group (χ2=10.749, p<0.001 and χ2=5.2877, p=0.015, respectively). Analyzing these indicators in patients treated with an FC regimen we also see the advantage of maintenance therapy in relation to the observation group (χ2=49.896, p=0.0001 and χ2=9.985, p=0.002, respectively). Comparative analysis of progression free survival (PFS) of CLL patients who received various regimens revealed a significant difference. Thus, in patients who completed the program followed by RFC and supporting rituximab therapy, the median PFS was achieved, while in patients without supporting rituximab therapy it was 42 months (p=0.009). The related indicators of patients receiving the combination of FC with further supporting rituximab therapy differed significantly: their median PFS was not achieved in contrast to patients in the monitoring group, whose PFS was 24 months (p=0.001). During the period of supporting rituximab therapy no additional toxicity was observed. Conclusion. The results of our study confirm the role of rituximab therapy in CLL remission maintenance.

0531

FLUDARABINE PLUS RITUXIMAB CHEMOIMMUNOTHERAPY FOLLOWED BY A CONSOLIDATION AND MAINTENANCE PLAN WITH RITUXIMAB IMPROVES OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA

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The treatment target of chronic lymphocytic leukemia (CLL) is the attainment of an optimal disease control combining chemotherapy with monoclonal antibodies. This approach may produce complete molecular remissions and longer response duration (RD), remaining often a minimal residual disease (MRD). We treated in first line 158 CLL symptomatic patients (pts), after informed consent, median age 65 years, with six monthly courses of intravenous (25 mg/sqm) or oral (30-40 mg/sqm) fludarabine and then, after a median time of 30 days, with four weekly doses (375 mg/sqm) of rituximab (txr). Fourteen pts had a modified low Rai stage, 121 an intermediate stage and 5 a high stage. The txr defines high risk pts having at least two of these markers: unmutated IgVH, CD38>30%, ZAP-70>20%, intermediate/unfavorable cytogenetics (trisomy 12 or del11q or del17p). For MRD flow cytometric study, the threshold was set at >1% CD19+CD5+CD79a+ bone marrow (BM) CLL cells. Based on NCI criteria 106/138 (77%) pts
achieved a complete remission (CR), 26/138 (19%) a partial remission (PR) and 6/138 (4%) stable disease or progression. Phenotypic CR (CD19+CD5-CD79b- BM cells <1%) was achieved in 80/138 (58%) CLL pts. Interestingly, MRD+ pts showed a significant shorter overall survival (OS) compared with MRD- pts (24% vs 72% at 16 years, P=0.0016). During the induction and consolidation/maintenance, 13 pts underwent grade 2-3 (WHO) infective lung toxicity and 2 pts progressed. R-FC’s syndrome was observed in all patients, usually mild including mainly neutropenia (grade 3 and/or 4 in 60 pts) and thrombocytopenia (grade 3 and/or 4 in 8 pts). Fifty-seven pts (43%) either in CR with B-CLL, BM cells >1% (MRD+, n=15 pts) or in CR MRD negative, but developing MRD positivity within 2 years after induction (n=24 pts) or in PR (n=18 pts), underwent consolidation and maintenance therapy with four monthly cycles of rtx at 375 mg/m²q follow by twelve monthly low doses of rtx (150 mg/m²). The median follow-up duration was 59 months. Noteworthy, both persistently MRD negative (≥2 years) pts (n=52) and pts undergoing consolidation/maintenance therapy (n=57) showed a longer RD vs MRD+ not consolidated pts (n=23) (76% vs 57% vs 0% at 5 years, P=0.0001). Equally, OS was shorter in MRD+ not consolidated pts in comparison with the other two subsets (0% vs 61% vs 97% at 15 years, P=0.05). Moreover, ZAP-70+ or unmutated IgVH pts revealed shorter RD (17% vs 53% at 16 years, P=0.001; 16% vs 55% at 6.5 years, P=0.0001). Importantly, within the high risk subset (n=59), pts were found persistently phenotypic CR (n=20) and consolidated pts (n=20) showed a longer RD (90% vs 61% vs 0% at 2.5 years, P=0.0009) vs MRD+ not consolidated pts (n=13). In multivariate analysis, consolidation/maintenance (<0.0001) and biological risk classes (P=0.001 and P=0.0001) were confirmed as independent prognosticators with regard to RD and OS. Therefore, persistent phenotypic CR and/or in CR MRD negative pts undergoing maintenance therapy improve RD and OS in CLL, also within the high risk subset, and important biological markers such as ZAP-70 and IgVH mutational status retain their prognostic impact with regard to the clinical outcome.

0532

A PHASE II STUDY OF CHLORAMBUCIL-RTUXIMAB (CLB-R) FOLLOWED BY R MAINTENANCE VS OBSERVATION IN ELDERLY PATIENTS WITH PREVIOUSLY UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): INDUCTION PHASE RESULTS


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47 patients enrolled. Median age was 70.0 years (range 61-84). Overall, 52.9% of patients were ≥70 years. One or more comorbidities were recorded at baseline in 47.1% of cases, Binet’s stage was A in 25.9% of cases, B in 57.6% and C in 16.5%. Trisomy 12, deletion 13q, deletion 11q and deletion 17 were found in 13.5%, 48.2%, 19.3% and 4.8% of cases, respectively. p53 mutations were recorded in 4.8% of patients. Fifty-eight percent were IGHV unmutated, 41% CD38+ and 75.9% ZAP-70+. The ORR was 81.2% (69 pts). CR, confirmed by CT scan, was found in 16.5% of pts (14 pts), CRi in 2.4% (2 pts), nPCR in 2.4% (2 pts) and PR in 60% (51 pts). In the 14 CR cases, MRD evaluated by flow cytometry and/or in blood and marrow was negative in 2; no patient was PCR positive. A treatment failure was recorded in 18.8% of cases (16 pts): PD (3.5% (3 pts), SD 4.7% (4 pts) and lack of response assessment 10.6% due to early treatment withdrawal (9 pts: investigator’s decision, 2 treatment-related AEs, 4 treatment-unrelated AEs, 5). Twenty-five AEs were recorded in 17 patients: 5 (4 pts) related to CLB only, 3 (3 pts: pleural effusion, 1 anemia, 1 neutropenia, 1) related to CLB-R and 12 (12 pts) treatment-unrelated. The most common hematologic toxicity was neutropenia (grade III-IV: 13.5% of patients, 7.4% of episodes and 2.9% of cycles). The median number of administered CLB-R cycles in patients <80 years was 6. A dose reduction of CLB was required in 7.8% of cycles, mainly for myelotoxicity. Conclusions: This study shows that R-CLB is an active and well tolerated front-line regimen for elderly CLL patients.
ceived alemtuzumab (median age 58yrs [40-77] and 35 [74.5%] males). There was a median of 2 prior therapies (range 1 to 4) with 46 patients receiving fludarabine combinations and 9 rituximab-containing combinations. There were a total of 22 SAE’s in 17 (36.2%) patients with 2 (4.3%) treatment related deaths (EBV-LPD and parainfluenza). G-CSF was given when the neutrophil count fell below 1 x 10^9/l, and 27 (58%) patients required G-CSF during or after alemtuzumab. Positive CMV PCRs were detected in 21 (45%) patients, all of whom were successfully treated with pre-emptive antiviral therapy. 13/23 (56%) patients in partial remission achieved a CR three months after alemtuzumab. 39/47 (83%) patients had MRD negative marrows at the end of therapy. 6/9 patients receiving 12 weeks of alemtuzumab became MRD negative, but only 1 (17%) responder remained MRD negative in the blood at 6 months. In contrast, of the 33 MRD negative patients after 6 weeks of treatment, 19 (58%) remained MRD negative at 12 months. Therefore MRD negativity in blood at 6 months predicts for persistent MRD negativity better than the marrow assessment at the end of therapy. 6/9 patients receiving 12 weeks of alemtuzumab became MRD negative, but only 1 (17%) responder remained MRD negative in the blood at 6 months. In contrast, of the 33 MRD negative patients after 6 weeks of treatment, 19 (58%) remained MRD negative at 6 months. The 24 month PFS after start of alemtuzumab consolidation is 82% for all patients and 85% for those who achieved MRD negativity. Of the 18 MRD negative patients 6 months after alemtuzumab, 15 (83%) remained MRD negative; Grade 3/4 AEs included thrombocytopenia (27%) and neutropenia (17%). One patient each had a serious, navitoclax-related AE: 1 patient with nausea (43%), both most likely attributable to the formulation; 2 patients had thrombocytopenia (32%) and neutropenia (17%). One patient each had a serious, navitoclax-related AE: pyrexia (Grade 1/2), tumor-lysis syndrome (Grade 3), dizziness (Grade 1/2). Three patients had AEs leading to discontinuation, and 9 leading to dose reduction, mainly thrombocytopenia. Nineteen patients discontinued: 6 due to PD, 6 due to AEs, 5 withdrew consent, and 2 due to other reasons (lack of response, investigator decision based on low-trending platelets). Two of 4 patients with 17p- achieved PR; patients with 11q- (n=5) appeared to have favorable outcome vs patients with 17p- (n=4) or with normal cytogenetics (n=4) (PFS 183 days vs not reached, p=0.0534). Conclusions: These data confirm that navitoclax has an acceptable safety profile at 250mg/day and significant anti-tumor activity in patients with heavily pre-treated CLL, including those with 17p- and other high-risk cytogenetic characteristics. Updated results will be presented.
Red cells

0535
THE CGMP PATHWAY AS A DRUG TARGET FOR THE REDUCTION
OF VASO-OCCLUSION IN SICKLE CELL ANEMIA MICE: ACUTE EFFECTS
OF HYDROXYUREA AND A PHOSPHODIESTERASE 9 INHIBITOR

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Background. Modulation in the levels of the second messenger cyclic guanosine monophosphate (cGMP) downstream in the nitric oxide (NO) pathway, represents a possible therapeutic approach for sickle cell disease (SCD). Up-regulation of cGMP-dependent signaling may induce γ-globin production in erythroid-lineage cells and in vitro experiments demonstrate a reduction in the adhesive properties of leukocytes following activation of this pathway. Evidence indicates that hydroxyurea (HU) may be highly expressed in hematopoietic cells, possibly providing a cell-specific drug target. Aim. Since leukocyte adhesion to the vessel wall plays a crucial role in vaso-occlusion initiation, we investigated the effects of the acute administration of HU and a PDE9-inhibitor on the vaso-occlusion process. Methods. Fully chimeric male sickle cell mice (SCD mice), produced by transplanting the bone marrow of Berkeley SCD mice to lethally irradiated C57BL/6 animals, were utilized for intravitreal microscopy at 3–5 months post-transplantation. An inflammatory process was induced in mice by TNF-α injection (0.5 µg i.p.); mice were concurrently treated with BAY73-6691 (3 mg/Kg i.v.) or HU (100 mg/Kg i.v) or both drugs or vehicle. At 2.5h after TNF-α administration, leukocytes were videoed continuously for 1 min. Leukocyte rolling, adhesion and extravasation were monitored and analyzed for 45 minutes after surgery. Results. SCD mice (N=4) treated with hydroxyurea and BAY73-6691 demonstrated increased leukocyte rolling (36.9±2.2; 14.1±3.9 leuk/min, for HU+BAY73-6691 and vehicle control, respectively, p<0.05). Reduced leukocyte adhesion to the vessel wall was observed in the treated groups (3.7±0.4; 3.7±0.7; 1.6±0.1; 6.0±0.3 leuk/100µm, for HU; BAY73-6691; HU+BAY73-6691 and vehicle control, respectively, p<0.0001; n ≥ 4). Cell extravasation was also decreased in all three groups (2.0±0.2; 1.1±0.1; 3.7±0.2; 3.2±0.4 leuk/100µm, for HU; BAY73-6691; HU+BAY73-6691 and vehicle control, respectively, p<0.0001; n≥4). Additionally, administration of BAY73-6691 or HU+BAY73-6691 decreased RBC-leukocyte interactions (0.5±2.0; 2.0±2.0; 0.7±0.4 RBC-leukocyte interactions/min, for BAY73-6691; HU+BAY73-6691 and vehicle control respectively, p<0.05). Surprisingly, the combination of drugs significantly prolonged the survival of SCD mice after TNF-α (p<0.05; n≥5). In C57BL/6 mice, where HU and BAY73-6691 also reversed TNF-α-induced alterations in leukocyte parameters, ODQ (guanylate cyclase inhibitor, 15mg/Kg i.v.) was able to reverse the effects of HU on leukocyte adhesion (3.2±0.2; 9.4±0.7 leuk/100µm, for HU and ODQ+HU, respectively, p<0.0001; n≥4). Similarly, when C57BL/6 mice were treated with KTS823 (protein-kinase-G inhibitor; 1mg/Kg i.v.) and BAY73-6691, the reduction in leukocyte adhesion was reversed (3.2±0.3; 8.8±0.8 leuk/100µm, for BAY73-6691 and KTS823+BAY73-6691, respectively, p<0.0001; n≥4). Conclusions. These results suggest that drugs that target the NO/cGMP pathway may reduce vaso-occlusive processes and increase survival, at least in SCD mice. Importantly, HU, thought to have NO donating properties, when administered acutely, was seen to significantly alter leukocyte properties in the SCD mouse, demonstrating that this drug could have immediate beneficial effects that are independent of its fetal hemoglobin-elevating properties. Furthermore BAY73-6691, a tissue-specific drug, when combined with HU amplifies the beneficial effects observed on the vaso-occlusion process.

0536
RELATIONSHIPS BETWEEN PLASMA NON-TRANSFERRIN-BOUND IRON AND MARKERS OF IRON OVERLOAD, ANAEMIA AND INEFFECIVE ERYTHROPOIESIS IN NON-TRANSFUSION-DEPENDENT THALASSAEMIA SYNDROMES

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Background. Patients with non-transfusion-dependent thalassaemia (NTDT), such as β-thalassaemia intermedia, milder forms of haemoglobin (Hb)E/β-thalassaemia and HbH α-thalassaemia, have little or no requirement for transfusions, but may still develop iron overload as a consequence of increased gastrointestinal iron absorption secondary to ineffective erythropoiesis. Factors determining the distribution of iron in NTDT are not completely understood. The prospective, randomized, double-blind, placebo-controlled Phase II clinical trial of deferasirox (THALASSA) enrolled 166 patients with NTDT and iron overload. Laboratory parameters prior to treatment initiation are analyzed. Aims. To assess the interrelationships between the key components of the NTDT phenotype, namely anaemia, ineffective erythropoiesis and iron overload. Methods. Patients aged ≥10 years with NTDT, liver iron concentration (LIC) ≥5 mg Fe/g dry weight (dw) and serum ferritin (SF) levels >800 ng/mL were enrolled into the study. Exclusion criteria included: anticipated requirements for regular transfusion during the study period, transfusion within 6 months or chelation therapy within 1 month prior to study entry, HbS variants of thalassaemia and impaired renal or liver function. Correlations were assessed by simple linear regression model. Results. Of 166 patients randomized, mean age was 32.1 ± 12.0 years and 55.6% were male. Most had β-thalassaemia intermedia (57.2%) or HbE/β-thalassaemia (29.5%), while the remaining 13.3% had HbH α-thalassaemia. 55.0% of patients were splenectomized. Patients generally had received little or no transfusion on a regular basis; 21 patients (12.7%) were not transfused at all. The randomized patients were anaemic (median Hb 8.1, range 4.5-14.0 g/dL) and iron-overloaded; the latter being reflected by increased SF (median 992, range 304-6419 ng/mL), LIC (median 12.1, range 2.6-49.1 mg Fe/g dw), transferrin saturation (TSAT; median 90.8, range 24-100%) and non-transferrin-bound iron (NTBI; median 2.2, range -3.2 to 8.5 µmol/L). There was a significant correlation between SF and LIC, which confirmed previous reports (Taher et al. Haematologica 2008). The patients showed increased levels of serum erythropoietin (EPO; median 101.0, range 18.3-3405.0 U/L), soluble transferrin receptor (sTfR; median 28.8, range 8.3-64.3 mg/L) and growth differentiation factor 15 (GDF15; median 9179, range 689-53,730 ng/L); the latter two are markers of ineffective erythropoiesis. sTfR showed a significant inverse correlation with Hb and a significant positive correlation with EPO, while GDF15 correlated significantly with sTfR and EPO. NTBI correlated significantly with TSAT, SF and LIC. A weaker but significant correlation was also found between NTBI and sTfR. Statistics for all correlations are presented in the Table. Interrelationships of underlying disease, age and previous therapy with the indices of iron metabolism and erythropoiesis will also be analyzed. Conclusions. Non-transfused or infrequently transfused NTDT patients develop significant...
iron overload as assessed by LIC and SF in parallel with increments in TSAT and NTBI, the latter of which is the source of potentially toxic free iron. Increased levels of NTBI result from both significant iron overload and ineffective erythropoiesis, two key components of the NTDT phenotype.

0537 RISK OF ACUTE ISCHAEMIC STROKE (AIS) IN CHILDREN WITH SICKLE CELL DISEASE (SCD) SCREENED WITH TRANSCRANIAL DOPPLER (TCD) PRE- AND POST-STOP PROTOCOL IMPLEMENTATION

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Background. The STOP trial, published in 1996, showed that regular transfusion of children with SCD and abnormal TCD velocities reduced the risk of AIS by about 90%. Aims. To assess the long-term results of TCD screening +/- early transfusion of children with abnormal TCD in a non-trial setting. Methods. We compared two cohorts of children followed in our network. Cohort 1 children were first screened with TCD between 1991 and 2000. Recomendations for transfusion were made in a joint haematology/neurology specialist clinic on the basis of symptoms and signs of ischaemia. Cohort 2 children were first screened after 1/1/2001 and were routinely offered transfusion for primary AIS prophylaxis if found to have abnormal TCD. All children had a diagnosis of HbSS or HbS/beta thalassaemia. Those with a previous stroke were excluded. Scans were done by FT, FK, BK and CA, using non-imaging equipment. Classification of abnormal scan required Vmax>200cms/sec on two occasions. Stroke was diagnosed by standard clinical criteria and confirmed by cerebral MRI. Follow-up for both cohorts continued either until censorship date of 29/2/2011, death or transfer to a different adult clinic at age 16. Kaplan Meier survivorship and Cox proportional Hazards modelling were used for data analysis. Results. 557 children had a total of 1548 scans. Cohort 1 consisted of 81, and Cohort 2 of 456 children. Average age at first scan was 7.2 years and did not differ between the two cohorts. The majority of children continued adult follow-up in our unit after the age of 16. Doppler categories at first scan were: Standard 78%; Conditional 12%; Abnormal 8%; Low velocity/asymmetric 1%; inadequate 6%. There was a significant difference in doppler categorization at first scan between the two cohorts, with relatively more conditional scans and less abnormal in Cohort 2. During follow-up, a total of 41 (7.6%) children developed abnormal Doppler. The risk of abnormal Doppler at 5,10, and 15 yrs of age was 2%, 8% and 12%. 5 (6.2%) in Cohort 1 developed abnormal TCD, and only one was transfused prospectively compared to 36 (7.9%) in cohort 2, the majority progressing from conditional to abnormal. 35 of these were transfused prospectively. There were a total of 11 (2%) AIS events, 5 (6%) in Cohort 1 and 6 (1.3%) in Cohort 2. The probability of remaining free of AIS at ages 5,10,15 and 20 years for Cohort 1 100%; 96.3%; 95.7% and 91.9%. For Cohort 2 they were 99.8%; 99.6%; 96.6%; 98.3%; 90.4%. There was a trend to reduced risk of AIS in Cohort 2, although this did not reach statistical significance (p=0.09) due to the small numbers with AIS. Conclusions. We showed a lower percentage developing abnormal TCD than previously described. Transfusion is generally accepted by parents of children with abnormal TCD. AIS rate is only 1.7% at age 15 in those managed according to the STOP protocol, but not completely prevented. There is some evidence for an advantage in prospective transfusion over transfusion based on clinical assessment in these children.

0538 HFE MUTATIONS ASSOCIATED WITH HIGH LEVEL SPORT PERFORMANCE

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Background. Iron is essential to erythropoiesis and muscular metabolism. High levels sport is associated with a lower iron biodisponibility due to higher iron absorption, sequestration or loss excess (sweat, microhemorrhage, hemolysis) that may impair athlete performance. Recently, the increase of hepcidin synthesis during sport training has been shown to lower iron bioavailability. Strong experimental evidence suggests that hemochromatosis associated to HFE mutation is due to a decrease in hepcidin production. Therefore, HFE mutations may counterbalance the decrease of iron availability and as such may improve performance in high level athletes. Aims. To assess HFE mutations frequency in elite athletes and its impact on sport performance. Methods. We performed a prevalence study of HFE gene mutations in sportmen of four top level French teams, practicing aerobic (Group 1, n=95: nordic ski, rowing), anaerobic (Group 2, n=34: judo), and non energetic (Group 3, n=41: petanque), compared to control subjects, matched by geographic origin, age, and gender (n=219). High level performance can be assessed through the titles and podiums collected during international competitions (continental or world championships and olympic games). In group 1, we compared athletes, who reached the three best places (international podium group: IPG, n=17) to those who did not succeed to reach this level (no international podium group, NPG, n=60). According to the Necker hospital Ethics committee, sport and control subjects have been screened for 18 haemochromatosis linked gene mutation. Test strip containing allele-specific oligonucleotide probes immobilized on the test strip were used. Results. We used logistic regressions to test the relative frequency of the mutations in athletes compared to the control group, and expressed as odds-ratio. Results. Among HFE mutations, H63D was found in 106 cases (27%), C282Y in 28 cases (7.2%), S65C in 11 cases (2.8%) and H687F in 1 case only (0.3%). Among them, 92% were heterozygotic. In energetic sports, the frequency of any mutation was superior to the control (Group 1: OR1=1.97, p=0.008, Group 2: OR2=3.85, p=0.003). In contrast, a non significant difference was found in the non energetic Group 3: OR3=1.12, p=NS). In the international podium group, the frequency of any mutation was even larger: among Group 1 athletes, the HFE mutation frequency was 13.3 higher in the subgroup with international titles as compared to the NPG (p=0.0001). The higher frequency of HFE mutations was found in both genders, with a tendency toward higher OR in women (OR1: 3.57, OR2: 3.92 vs 2.57, OR3: 9.42 vs 2.57, for women and men respectively). Conclusion. This study demonstrates that HFE frequency is significantly larger in French athletes of high energetic sports and strongly correlated with international top performance. During evolution, these genotypes altering a major protein
of iron, red cell and muscle metabolism may have been selected, in the heterozygotic form, due to their large impact on phenotypes ie. energy performance under extreme physiological constraint. Genetic studies in high level sport might reveal such associations.

**0539**  
**GLOBIN GENE EXPRESSION IS CORRELATED WITH G PROTEIN-RELATED GENES DURING ERYTHROID DIFFERENTIATION**

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The pattern of changes in human globin gene expression during development has been well studied. To study the contributions of other genes and pathways to these processes, we have used oligonucleotide microarray and real-time quantitative PCR technologies to examine gene expression modulation in human erythroid progenitor cells of various tissues during ontogeny. Human hematopoietic CD34+ progenitor cells were isolated from fetal liver (FL), cord blood (CB), adult bone marrow (BM), peripheral blood (PB) and G-CSF stimulated mobilized PB (mPB), and then differentiated in vitro into erythroid progenitor cells (EPC) by cocktail of cytokines including erythropoietin. We found that cell growth capacity was most abundant in FL and CB-derived cells. The EPC were sorted as 100% CD71+. During ontogeny, beta-globin gene expression reached maximum levels in cells of adult blood origin (176 fmol per microgram of RNA). For the period of early in vitro erythropoiesis culture, gamma-globin gene expression was consistently up-regulated in CB-derived cells (60 fmol). In microarray studies, a total of 3917 genes were persistently expressed in mPB, 3844 in CB, 1770 in BM, 1755 in FL and 1325 genes in PB-derived EPC. A total of 994 common genes were identified in majority of samples of EPC determined for 1-year (latest observation). Western blot detected NPMc+ protein in peripheral blood, spleen and BM cells. After Cre induction transcription expression was maintained for 1-year (latest observation). Western blot detected NPMc+ protein in peripheral blood, spleen and BM cells and demonstrated the NPMc+ mutant localized in the BM cell cytoplasm. Strikingly, compared with NPMc+MxCre- controls, platelet counts were significantly lower while white blood cells counts and hemoglobin levels were not. Platelets were one-half of the control count in mutants harboring one conditional allele and one-quarter in homozygous NPMc+ knock-in mice. To investigate whether the low platelet count was due to abnormal hematopoiesis, 2 months after Cre induction we analyzed BM cells from NPMc+MxCre+ mice. Results. In NPMc+MxCre+ mice RT-PCR detected NPMc+ transgene mRNA in peripheral blood, spleen and BM cells. After Cre induction transgene expression was maintained for 1-year (latest observation). Western blot detected NPMc+ protein in peripheral blood, spleen and BM cells and demonstrated the NPMc+ mutant localized in the BM cell cytoplasm. Strikingly, compared with NPMc+MxCre- controls, platelet counts were significantly lower while white blood cells counts and hemoglobin levels were not. Platelets were one-half of the control count in mutants harboring one conditional allele and one-quarter in homozygous NPMc+ knock-in mice. To investigate whether the low platelet count was due to abnormal hematopoiesis, 2 months after Cre induction we analyzed BM cells from NPMc+MxCre+ mice. Compared with controls, CD41+ cells were double in number (p<0.05) while serum TPO levels were identical. Lin-Kit+Sca-1-CD150+CD41+ megakaryocytic progenitors (MkPs) were increased 2-fold (p<0.001), suggesting NPMc+ expression leads to an expansion of immature megakaryocytes. Interestingly, immunohistochemistry on BM trephines from patients with NPMc+ AML detected megakaryocyte expansion in some. No significant differences emerged in total BM cellularity, in Lin-Kit+Sca-1+ cells or Lin-Kit+Sca-1-CD41-CD150+FeR-CD105lo erythromegakaryocytic progenitor cells. The splenic CD41+ cell count and the spleen/body weight ratio were higher (p<0.05 for both). Since miR-10a down-regulation was recently hypothesized to unblock target genes involved in megakaryocytic differentiation, we evaluated miR-10a expression in BM cells. Compared with controls, mi-R10a was significantly up-regulated, suggesting the increase in MkPs was related to a differentiation blockade. MiR-10a up-regulation was associated with increased expression of HOXB4 and HOXB5 genes in which cluster miR-10a is embedded. Similar findings have already been described in human NPMc+ AML. There was no leukemic evolution after 1 year follow-up. Summary: These results demonstrate that NPMc+ expression impedes megakaryocyte maturation by blocking differentiation. This new mouse model is expected to aid understanding of the molecular and genetic background to NPMc+ AML.
DNMT3A MUTATIONS IN ACUTE MYELOID LEUKEMIA: RESULTS ON 687 PATIENTS TREATED WITHIN THE AML HD98A STUDY OF THE AML STUDY GROUP (AMLSG)

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Background. Alteration of DNA methylation is a hallmark of epigenetic modification in human cancers. A gene family of DNA methyltransferases (DNMT), DNMT3A, DNMT3B and DNMT1b, catalyze the addition of a methyl group to the cytosine residue of CpG dinucleotides affect promoter methylation status and therefore gene expression. Using a next generation sequencing approach a frameshift mutation of DNMT3A has been detected in an AML case. Subsequent sequencing analysis in an independent cohort of 288 AML patients (pts) revealed DNMT3A mutations in 22% of the pts; DNMT3A mutations were associated with intermediate-risk cytogenetics and poor outcome. Aims. To evaluate frequency and clinical impact of DNMT3A mutations in younger (16 to 60 years of age) adult AML pts who were treated within the AML HD98A study. Methods. DNMT3A mutation screening was performed in 687 AML (de novo AML, n=631; s-pact of s-pact of RUNX1-M0, n=56) with RUNX1 allele (CIR) and overall survival (OS) in the whole AML cohort (P=.0541). Results. DNMT3A mutations were found with an overall incidence of 18% (125/687), with two AML exhibiting two mutations. 109 mutations were located in the MTase domain clustering at amino acid R882 (77%). All mutations were heterozygous. DNMT3A sequence alterations included 7 frameshift, 3 nonsense and 117 missense mutations. Pts with DNMT3A mutations were significantly older (P=.009), had higher white blood cell and platelet counts (P<.001, P=.001, respectively) and higher LDH serum levels (P=.06, respectively). There was no correlation with respect to type of AML (P=.28, respectively). Conclusions. We confirm that DNMT3A mutations are frequent genetic aberrations in AML, associated with normal karyotype, and NPM1, FLT3-ITD and IDH1 mutations. Our data suggest a negative prognostic impact in molecular-risk high CN- AML, however, these data need to be validated in larger patient cohorts.

OVEREXPRESSION OF SET IS A RECURRENT EVENT ASSOCIATED WITH POOR OUTCOME THAT CONTRIBUTES TO PP2A INHIBITION IN ACUTE MYELOID LEUKEMIA

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Protein phosphatase 2A (PP2A) is a tumor suppressor reported as a potential therapeutic target in chronic and acute leukemias. The protein SET (22P2A/TAF-Iβ), a potent PP2A inhibitor, has been implicated in many cell processes and signaling pathways. Moreover, SET has been described as an oncogene that is overexpressed in several neoplasms, including chronic myeloid leukemia. Here we report that SET overexpression in AML cells. In addition, we postulated that SET deregulation could be one mechanism contributing to the inhibition of PP2A activity, which in turn could lead to drug resistance, cellular proliferation in AML cells. In order to study whether SET was deregulated in AML, we first analyzed its levels in 13 AML cell lines, observing SET overexpression at both mRNA and protein levels. Prevalence of SET overexpression in AML patients at diagnosis was 28% (60/214), and was associated with SETBP1 (p<0.01) and EVI1 overexpression (p=0.02). Interestingly, patients with SET overexpression had worse overall survival (p<0.01) and event free survival (p<0.01). We next confirmed SET overexpression at protein level in a series of 16 patients with AML at diagnosis. We found increased levels of SET protein in 9 out of 16 cases (56.2%), although only 6 of these had SET overexpression by real-time RT-PCR (QRT-PCR), which indicates that SET overexpression is not a frequent event in AML that could be underestimated by QRT-PCR. It has been demonstrated that SET upregulation, and the resulting PP2A inhibition, is critical in BCR/ABL-positive cells to fulfill its tumorigenic potential. Analysis by MT5 assay showed that ectopic expression of SET restores proliferation in AML cells ectopically expressing PP2A. When we investigated the molecular mechanisms involved in SET deregulation in AML, we observed that activation of PP2A leads to reduced SET levels. Therefore, we postulated that the inhibited status of PP2A could contribute to deregulate SET in AML cells. Moreover, analysis of the SET proximal promoter identified two positive regulatory elements such as AP-1, GATA1, and EVI1. In our series of AML patients, SET and EVI1 overexpression were associated, suggesting that EVI1 could regulate SET. ChIP showed that EVI1 binds the SET promoter; however, we detected no differences in the luciferase assay, sug-

MODELING RUNX1 BIALLELIC MUTATIONS ASSOCIATED WITH AML-M0 AND AML-FPD IN MICE REVEALS IMPORTANCE OF RESIDUAL RUNX1 FUNCTION

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Disruption of the RUNX1 gene is one of the most common aberrations found in myeloid leukemia. The most common disruptions are chromosomal translocations, but intragenic mutations are found at a high incidence in minimally differentiated AML (AML-M0) and in AML secondary to familial platelet disorder (AML-FPD). The majority of these mutations impact the conserved Runx domain, specifically disrupting the DNA-binding (DB) interface, whereas other mutations are predicted to be null alleles, due to complete deletion or frameshifts leading to premature stop codons. Interestingly, although monogenic RUNX1 biallelic mutations have been reported in 27/28 families reported with FDP, recent evidence demonstrates that patients developing AML have acquired mutations in RUNX1 or RUNX1-RUNX1-DB. These data also show a high incidence of biallelic RUNX1 mutations (circa 25%). In both cases, trisomy 13 (correlating with high FLT3 expression) or FLT3 activating mutations (FLT3-ITD) are common secondary mutations. Earlier work by us and others have shown that various functional inactivation of the RUNX1 gene or expression of DB-RUNX1 mutants leads to increased self-renewal capacity of myeloid progenitors in vitro. We thus predicted that either null or DB mutations may disrupt a critical ‘gate-keeper’ function of this gene, permitting the accumulation of secondary mutations (e.g. FLT3 activation) that lead to an overt leukemia. In a study to test our hypothesis, constitutively active FLT3-ITD alone or together with RUNX1-DB was introduced into hematopoietic progenitors from RUNX1-deficient, heterozygous, or wildtype mice. These studies showed that in the C57Bl/6 mouse background, activated FLT3-ITD most readily induces a T-cell thymoma. Neither RUNX1 inactivation nor coexpression of RUNX1-DB shifted the disease spectrum to myeloid neoplasia. However, together, FLT3-ITD, RUNX1 deletion, and RUNX1-DB led to a rapid development of an AML-like myeloid disorder. Therefore, altered RUNX1 function may support a tumor suppressor function for wildtype RUNX1 but indicate that RUNX1 DB mutants have retained important RUNX1 oncogenic activity, which cooperates with loss of wild-type RUNX1 in disease progression. Important signaling pathways differentially regulated by wildtype RUNX1 vs. DB-RUNX1 will be discussed. These results highlight the significance of the specific mutations in the DNA-binding interface and the high incidence of biallelic mutations in AML-M0 and AML-FPD.
gesting that EVI1 could regulate SET indirectly. In summary, we demonstrate that SET overexpression is a recurrent molecular event associated with poor outcome in AML, which promotes cell proliferation and restores the reduced cell viability induced after PP2A overexpression. Moreover, PP2A activation status could be involved in the regulation of SET. Altogether, SET overexpression could differentiate a subgroup of patients with poor prognosis who could be treated with PP2A activators in future clinical trials. e-mail address: icristobal@alumni.unav.es

0544

SLEEPING BEAUTY DRIVEN LEUKAEMOGENESIS FOLLOWS AN ACCELERATED DARWINIAN-LIKE EVOLUTION IN A MOUSE MODEL OF NPM1C+ ACUTE MYELOID LEUKAEMIA

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Heterozygous somatic mutations in NPM1, the gene for Nucleophosmin, are the commonest group of mutations in acute myeloid leukaemia (AML). We recently described a humanised mouse model of these mutations, in which one third of mice with conditional activation of a humanised Npm1c knock-in allele, Npm1flox-cA, developed late-onset AML. The same model was subjected to insertional mutagenesis with Sleeping Beauty, through the mobilisation of 80 copies of the novel transposon GrOnc, from a resident locus on mouse chromosome 19. Rapid onset AML developed in 80% of these mice in association with recurrent insertions in many known and novel leukaemia genes including Csf2, Flt3 and Nup98 (Vassiliou et al, Nature Genetics 2011 - In Press). Here we report the findings from a similar but novel model mobilising 15 copies of GrOnc from mouse chromosome 16 (Figure 1a). Common integration sites (CISs) from the analysis of 40 leukaemias showed a striking overlap with CISs identified in the aforementioned study, confirming the strong cooperativity of such insertions with Npm1c (Figure 1b). To understand the molecular basis of leukaemogenesis in this model, fortnightly blood samples were taken from 12 of these mice from the time of activation of the SB transposase (via Mx1-Cre) to the onset of frank leukaemia. Additionally, single-cell derived leukaemic blast colonies were generated and analysed. Leukaemia onset was sudden and could not be predicted in advance from FBC parameters (Figure 1c). A subset of transposon integrations were found to occur early and persist over several months during leukaemia development (Figure 1d). Large numbers of transposon integrations (50-200) were identified within each tumour, however only a small number of these were common to multiple single cell colonies generated from the same tumour. Similarly only a few integrations persisted on serial transplantation of tumour cells. These results reveal that transposon mobilisation continues throughout leukaemia evolution leading to the development of multiple sub-clones within these neoplasms. Our data suggest that only a subset of the integrations identified in leukaemia samples behave as “driver”mutations, whilst most insertions are “passengers”. Continued mobilisation of transposons from these passenger sites occurs without loss of proliferative potential. By contrast, mobilisation of driver insertions in host cells is immediately selected against. Our findings validate the critical pathways able to cooperate with Npm1c in leukaemogenesis, whilst also giving important novel insights into the way Sleeping Beauty operates in carcinogenesis and highlighting critical differences to retroviral mutagenesis. We are currently applying these insights to improve recognition of leukaemic “drivers” and to develop novel applications, such as the identification of genetic pathways able to overcome inhibition of leukaemic growth by anti-leukaemic drugs.
The incidence of acute GVHD in B-cell lymphomas

In the last GITMO RIC-alloSCT trial we observed an incidence of grade II-IV acute graft-versus-host disease (GVHD) of 35% (Leukemia 2007). The present GITMO study is a prospective multicenter phase II trial designed for patients affected by CD20 positive lymphomas. It increases the thiotepa dose of 20%, and incorporates high-dose Rituximab (R) in a RIC regimen to improve the overall response possibly modulate the incidence of Chronic GVHD. Aims. Primary end-point was 1-year progression-free survival; secondary endpoints were non-relapse mortality and incidence of acute and chronic GVHD. Methods. Fifty-two patients were enrolled so far in the study and 34 are evaluable for preliminary analysis. Treatment plan consisted of high-dose R (500 mg/m^2 on day -6) followed by a RIC regimen containing thiotepa (12 mg/kg), fludarabine (120 mg/m^2) and cyclophosphamide (60 mg/kg). Graft-versus-host disease (GVHD) prophylaxis included cyclosporine and mini-methotrexate; ATG was added to the patients allografted from class I antigen mismatched sibling or unrelated donors. Histopathological subtypes included aggressive (n=11 diffuse large B-cell lymphomas, n=5 mantle cell lymphomas) and indolent lymphomas (n=10 follicular lymphomas, n=9 small lymphocytic/chronic lymphocytic leukemia). Patients were allografted from matched related siblings (n=25) or alternative donors (n=11). All the patients had chemosensitive disease (58% in complete remission) and 16 (47%) failed a previous autoSCT. Results. The median follow-up is 1 year (range, 180-1000 days). The cumulative incidence (CI) of non-relapse mortality (NRM) was 9% at 1 year. In total only 5 of 34 patients had acute GVHD (n=4 grade II, n=1 grade III) with an estimated CI of 17% at 100 days. Only 25 patients are evaluable for chronic GVHD with an estimated CI of 41% at 1 year (n=0 limited, n=2 extensive). Infection after engraftment requiring hospitalization or intravenous treatment occurred in 15 patients (44%). Preliminary data on immune-reconstitution showed absence of circulating CD19 B cells at 6 months after allograft. The CI of relapse was 19% and 31% at 6 months and 1 year, respectively. In the indolent and aggressive groups, OS estimates were 88% (95% CI, 60% to 97%) and 59% (95% CI, 31% to 79%) and PFS estimates were 63% (95% CI, 32% to 83%) and 55% (95% CI, 29% to 75%), respectively. Conclusions. The present data suggest that the administration of high-dose R is feasible and causes an unexpected reduction of the incidence of acute GVHD (only 1 case had GVHD grade III) without increasing the NRM and the incidence of severe infections after allografts. Complete blood data evaluating the effects of R on immune reconstitution are ongoing.

0547 IMMUNE-ABLATIVE REGIMEN FOLLOWED BY AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR NEWLY-DIAGNOSED TYPE I DIABETES

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3Background. Patients with Type I diabetes mellitus (DM) is mostly juvenile, insulin-dependent and associated with auto-immune mediated in nature. Aim. To determine the safety and efficacy of immune- ablative regimen followed by autologous hematopoietic stem cell transplantation (Auto-HSCT) in newly-onset type I DM patients. Methods. We conducted a prospective clinical trial in newly-diagnosed type I DM patients(NCT0107651). All patients received cyclophosphamide (200mg/kg) and ATG (4.5mg/kg) followed by infusion of autologous mobilized peripheral hematopoietic stem cells. Monitoring of serum hemoglobin A1c, C-peptide levels and anti-glutamic acid decarboxylase antibody (GAD) titers was carried out before and after Auto-HSCT. Results. A total of 18 patients were enrolled with a median average age of 18 (range, 15-25). The median follow-up was 414 days (range, 140-750). Among these patients, 12 (67%) patients achieved full stop of insulin with a median of 6 weeks (range 2-21) after HSCT. Four cases eventually resumed the insulin therapy all triggered by mild illness (common cold). With the last follow-up, 44.4% (8/18) remained free of insulin therapy, and the other patients achieved reduction of insulin dose on an average of 67.3% ± 22.4%. All 18 patients achieved a significant decrease of GAD level and among them 6 (33.3%) became negative. Fasting C peptide and postprandial 2 hour C peptide levels increased significantly after HSCT and the C peptide area under the curve (AUC) increased remarkably and can maintain for more than 1 year. Between the transplantation, 2 patients died. All patients had varying degrees of gastrointestinal reactions, hair loss, fever and bone marrow suppression. Five patients must received supportive blood transfusion. No severe adverse event involving the heart, liver, kidney and other or-

0546 HIGH-DOSE RITUXIMAB IN THE CONDITIONING REGIMEN BEFORE ALLOGENEIC STEM CELL TRANSPLANTATION REDUCES THE INCIDENCE OF ACUTE GVHD IN B-CELL LYMPHOMAS

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Background. Allogeneic stem cell transplantation (alloSCT) with a reduced-intensity conditioning (RIC) is an effective salvage therapy for relapsed lymphomas. However, in the last GITMO RIC-alloSCT trial we observed an incidence of grade II-IV acute graft-versus-host disease (GVHD) of 35% (Leukemia 2007). The present GITMO study is a prospective multicenter phase II trial designed for patients affected by CD20 positive lymphomas. It increases the thiotepa dose of 20%, and incorporates high-dose Rituximab (R) in a RIC regimen to improve the overall response possibly modulate the incidence of Chronic GVHD. Aims. Primary end-point was 1-year progression-free survival; secondary endpoints were non-relapse mortality and incidence of acute and chronic GVHD. Methods. Fifty-two patients were enrolled so far in the study and 34 are evaluable for preliminary analysis. Treatment plan consisted of high-dose R (500 mg/m^2 on day -6) followed by a RIC regimen containing thiotepa (12 mg/kg), fludarabine (120 mg/m^2) and cyclophosphamide (60 mg/kg). Graft-versus-host disease (GVHD) prophylaxis included cyclosporine and mini-methotrexate; ATG was added to the patients allografted from class I antigen mismatched sibling or unrelated donors. Histopathological subtypes included aggressive (n=11 diffuse large B-cell lymphomas, n=5 mantle cell lymphomas) and indolent lymphomas (n=10 follicular lymphomas, n=9 small lymphocytic/chronic lymphocytic leukemia). Patients were allografted from matched related siblings (n=25) or alternative donors (n=11). All the patients had chemosensitive disease (58% in complete remission) and 16 (47%) failed a previous autoSCT. Results. The median follow-up is 1 year (range, 180-1000 days). The cumulative incidence (CI) of non-relapse mortality (NRM) was 9% at 1 year. In total only 5 of 34 patients had acute GVHD (n=4 grade II, n=1 grade III) with an estimated CI of 17% at 100 days. Only 25 patients are evaluable for chronic GVHD with an estimated CI of 41% at 1 year (n=0 limited, n=2 extensive). Infection after engraftment requiring hospitalization or intravenous treatment occurred in 15 patients (44%). Preliminary data on immune-reconstitution showed absence of circulating CD19 B cells at 6 months after allograft. The CI of relapse was 19% and 31% at 6 months and 1 year, respectively. In the indolent and aggressive groups, OS estimates were 88% (95% CI, 60% to 97%) and 59% (95% CI, 31% to 79%) and PFS estimates were 63% (95% CI, 32% to 83%) and 55% (95% CI, 29% to 75%), respectively. Conclusions. The present data suggest that the administration of high-dose R is feasible and causes an unexpected reduction of the incidence of acute GVHD (only 1 case had GVHD grade III) without increasing the NRM and the incidence of severe infections after allografts. Complete blood data evaluating the effects of R on immune reconstitution are ongoing.
Table 1. Patient characteristics and outcomes.

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<tr>
<th>Characteristic</th>
<th>MM (n=40)</th>
<th>NHL (n=19)</th>
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<tr>
<td>Gender (male:female)</td>
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<td>11:8</td>
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<tr>
<td>Age (years)</td>
<td>Mean 58 (20-74)</td>
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<td>No. of patients with prior stem cell transplant</td>
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<td>No. of patients with prior autologous HSCT</td>
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<td>No. of patients with prior allo-HSCT</td>
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Acute lymphoblastic leukemia - Clinical

**0850**

IN ACUTE LYMPHOBlastic LEUKEMIA An MRD-GUIDED STRATEGY IMPROVES RISK STRATIFICATION, ABROGATES REMISSION MORTALITY IN MRD-NEGATIVE GROUP AND CONFIRMS THE ROLE OF TRANSPLANTATION IN MRD-POSITIVE GROUP


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Introduction. Despite major improvements achieved in the treatment of childhood ALL, significant challenges still remain. Traditional risk factors have proven to be important in predicting patient outcome, but more than half of dismal events still occur in non high risk patients. If identified early, these patients may benefit from an intensiﬁed treatment. However, current strategies fail to identify these patients and, in the case studied for new prognostic markers. Methods: We analyzed 245 patients with de novo ALL divided into training and validation cohorts. The training cohort consisted of 165 patients from the PETHEM Spanish group. The validation cohort comprised 80 patients from SHOP Spanish group trials. Protocols were approved by the IRB of the involved institutions and written informed consent was provided. We studied the methylation proﬁle of 50 genes (36 cancer-related genes and 14 microRNAs) belonging to pathways involved in cell transformation in the training cohort by MSP (Methylation-speciﬁc PCR) in order to design a Prognosis Index Score (PIS). Univariate and multivariable analyses were performed using the Cox proportion hazards model. The multivariable analyses were undertaken with both forward and backward stepwise procedures for identifying the independent prognostic variables. The model results were used to deﬁne levels of risk for survival. Results. Promoter methylation of 27 genes was found in at least 20% of the patients, and these genes were selected for further analysis as putative candidates. The methylation of 16 genes was associated (P≤0.1) with shorter OS. In multivariate analysis, methylation of 6/16 genes remained as independent predictors of OS (hsa-mir-196-2, Wnt5a, Reprimo, Wif1 and Lats1). ALL patients were classiﬁed into two different methylation phenotype (MP) groups: MP-negative (no methylated genes) and MP-positive (at least, one methylated gene). In addition, univariate analysis revealed four clinical variables (WBC count, presence of TEL-AML1, age at diagnosis and immunological phenotype) and the MP that were signiﬁcantly associated with OS. However, multivariable analysis showed that only three of these variables were independently associated with survival (age, MP and immunophenotype) and they were used to design a model to predict an individual patient’s risk of OS. The index score was deﬁned as the sum of the number of risk factors present with each risk factor receiving a value of 1 except for MP status, which was scored as 2 for MP-positive patients. Patients could receive a score from 0 to 4 and they could be grouped as low risk (score 0-1), intermediate risk (score 2, n=72), high risk (score 3, n=39) or very high risk (score 4, n=8). The mean survivals for these groups were 202.6, 150.2, 80.3 and 33.3 months, respectively, (P<0.0001). These four groups also showed distinctive differences in, relapse rate, mortality rate and DFS. Results were validated in an independent cohort of 80 patients. Conclusion. We have created a new prognostic score for ALL children that integrates for the ﬁrst time, traditional parameters and epigenetic data. Our scoring system stratifies patients into 4 groups at very different risk of death after treatment.
CLINICAL ACTIVITY OF THE ANTI-CD19 BITE BLINATUMOMAB IN PATIENTS WITH RELAPSED/REFRACTORY B-PRECURSOR ACUTE LYMPHOCYTIC LEUKEMIA (ALL): INTERIM RESULTS OF A PHASE I STUDY

M Topp,1 N Goebuget,2 G Zugmaier,1 A Vardot,6 M Steljes,5 S Neumann,1 HA Horst,1 A Reichle,6 R Marks,1 P Klappers,6 N Mergen,1 D Nagorsen,1 P Kufer,1 M Goebeler,1 D Hoelzer,2 R Bargou2

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Background. Relapsed/refractory B-precursor ALL is an aggressive malignant disease with a dismal prognosis and therapy is an unmet medical need. 80-90% of adults with relapsed ALL die from their disease or adverse effects of therapy. CD19 is the most frequently expressed B-cell differentiation antigen and can be targeted by blinatumomab, a member of a novel class of T-cell engaging, bispecific single-chain antibodies called BiTE antibodies. We initiated an open-label multi-centre exploratory Phase 2 study in collaboration with the German Multicenter Study Group for Adult Lymphoblastic Leukemia (GMALL) in order to determine the efficacy and safety of Blinatumomab in adult patients with relapsed/refractory B-precursor ALL. Methods. The primary endpoint is complete remission (CR) rate with hematological recovery. Secondary end-points are minimal residual disease (MRD) response rate (defined by an MRD level below the quantitative detection limit of 10e-4), time to hematological relapse and overall survival. Eligible patients must have B-precursor ALL re-lapsed after at least induction and consolidation or primary refractory disease. Prior allogeneic HSCT is permitted. Patients with Ph-positive ALL have to be ineligible for tyrosine kinase inhibitors. Blinatumomab administration as a 4-week continuous intravenous infusion followed by a 2-week treatment-free period. Responders may receive up to 3 additional cycles. The first cohort of five evaluable patients received a dose of 15 μg/m²/d. A second cohort receives 5 μg/m²/d for the first 7 days of the first cycle followed by 15 μg/m²/d for the remaining 5 weeks of the cycle and the following cycles. A risk-benefit assessment will determine which of the dose levels will be evaluated in the second stage enrolling 10 additional patients. Results. Seven patients have been treated in the first cohort. Their age ranged from 18 to 77 years. Four of the five evaluable patients had a reduction of bone marrow blasts < 5% within the first cycle two with CR and two with a complete remission with only partial hematologic recovery (CRh*). To date, three also have an elimination of MRD below quantitative detection limit within the first 2 cycles. One responder had an extra-medullary relapse during the third cycle of treatment. The most common adverse events were fever and chills. Two patients were early evaluated for remission as they had to permanently discontinue treatment without completion of the first cycle due to adverse events. One non evaluable patient with high leukemic burden had a completely reversible SAE of cytokine release syndrome (CRS). Subsequent patients with high leukemia burden were managed by pre-treatment with dexamethasone and/or cyclophosphamide, and no further treatment discontinuations due to CRS were observed. The second non evaluable patient had treatment discontinued due to a completely reversible CNS event of encephalopathy and disorientation. Despite a limited course of treatment, this patient showed an elimination of MRD below quantitative detection limit. Recruitment of the second cohort receiving a low initial dose of blinatumomab is ongoing. Conclusion. These initial data show that blinatumomab elicits pronounced anti-leukemic activity in patients with relapsed/refractory ALL and support further evaluation in this patient population.

ACUTE LYMPHOBLASTIC LEUKEMIA (ALL): INTERIM RESULTS OF A PHASE I STUDY

0553

CD20 EXPRESSION IN PHILADELPHIA-NEGATIVE B-CELL PRECURSOR ACUTE LYMPHOCYTIC LEUKEMIA DOES NOT SHOW SIGNIFICANT IMPACT ON OUTCOME: RESULTS OF NORTHERN ITALY LEUKEMIA GROUP ALL 09-2000 PROTOCOL

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Background. The prognostic significance of CD20 expression in B-cell precursor acute lymphoblastic leukemia (BCP-ALL) has been investigated in children and adults and its role is still under debate. The first study addressing the prognostic relevance of CD20 in adults was carried out by Thomas et al (Blood 2009); CD20 positivity (i.e. more than 20% of positive ALL cells) was associated with worse disease-free survival (DFS) and overall survival (OS), and this effect appeared unrelated to age. The French group (Maury et al, Haematologica 2010) documented a significant prognostic impact of CD20 only in patients with white blood cell (WBC) count above 30 x 10^9/L. Conversely, Chang et al (Haematologica 2010) did not detect any prognostic relevance of CD20. Aims. The aim of our study was to correlate CD20 expression with clinical-biological characteristics and outcome in Philadelphia-negative (Ph-) BCP-ALL patients prospectively treated within the multicenter NILG 09-2000 study (Bassan et al, Blood 2009), designed to orientate post-remission strategy upon minimal residual disease (MRD) assessment. Methods. Immunophenotyping was performed according to the general recommendations from European Group for the Immunological characterization of Leukemias (EGIL). Phenotypic data were expressed as the percentage of CD20 positive cells on whole leukemic population; we considered 20% as the threshold for positivity. Results. From March 2000 to September 2008, 172 Ph- BCP-ALL were enrolled in the study. Median age was 37 years (range 16-68); median WBC was 10 x 10^9/L (0.5-730). According to EGIL classification, BCP-ALL diagnoses were B-I 50, B-II 96, and B-III 26. Fifty-two (30.2%) patients resulted CD20-positive. The CD20-positive group showed
higher frequency of B-1 phenotype (77.0% vs. 33.3%; p<0.0001), and higher incidence of splenomegaly (53.8% vs 35.0%, p=0.067) and hepatomegaly (46.2% vs 25.8%, p=0.014), while no other difference was detected with regard to demographic and diagnostic characteristics. As regards treatment, no difference emerged between the two groups with respect to complete remission rate, MRD response, DFS and OS (Figure 2A). The prognostic impact of CD20 expression within specific patient and disease subgroups: age, WBC count and EGIL classification were not associated with CD20 positivity and CD20 expression did not affect outcome in any of these subsets. Exclusion of (t4;11)+ ALL from analysis did not alter these results. Summary/conclusions. Our study failed to demonstrate a prognostic significance for CD20 expression in BCP-ALL. The discrepancy between our data and others might be related to differences in study design and therapeutic strategy, herein MRD-oriented, that could have resulted in abrogation of the pejorative prognostic effect by CD20. Nonetheless, independently of these considerations, CD20 antigen remains a useful therapeutic target to improve outcome further in CD20+ ALL.

**0554**

**MINIMAL RESIDUAL DISEASE IN PERIPHERAL BLOOD AT DAY 15 PREDICTS PROGNOSIS OF CHILDHOOD B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA AND REFINES RISK STRATIFICATION BASED ON BONE MARROW**

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**Background.** Most minimal residual disease (MRD)-directed treatment interventions in current treatment protocols for acute lymphoblastic leukemia are based on bone marrow testing, which is a consequence of previous reports showing the superiority of bone marrow (BM) over peripheral blood (PB) as an investigational material. Those studies typically did not explore the prognostic impact of peripheral blood involvement and lacked samples from very early time points of induction therapy. Aims. To compare MRD levels in BM vs. PB at early time points of treatment and to evaluate their impact on prognosis. Design and Methods. In this study, we analyzed 398 pairs of simultaneously taken blood and marrow follow-up samples from 95 children with B-cell precursor acute lymphoblastic leukemia during ALL. BM-PB 2002 treatment using immunoglobulin and T-cell receptor gene rearrangement tests for diagnosis (n=95), day 8 (n=83) and day 15 (d15, n=76) of induction, the end of induction phase 1 - day 33 (n=53), pre-consolidation - week 12 (n=47), prior to maintenance therapy (n=6), and at the end of maintenance therapy (n=38). Results. Also at early treatment time points, we confirmed the previously published poor correlation between MRD in BM and PB, with BM-MRD being higher than PB-MRD in most samples (BM/PB: median 7.9, range 0.04-3 293). Higher PB involvement at diagnosis was associated with higher WBC (p=0.003), enlargement of the spleen (p=0.0004) and of the liver (p=0.05). No obvious difference has been observed between PB-MRD levels regarding immunophenotype or genetic subtype including Ikaros gene status, except for the fact that hyperdiploid leukemias had a trend towards lower d15 PB-MRD values than other patients excluding TEL/AML1 cases (p=0.057). At day 15, PB-MRD lower than 10-4 was achieved in 45% of patients and was associated with an excellent five-year relapse-free survival (100% vs. 69±7%; p=0.0008). PB-MRD subgroups (high-risk, HR=a10-2, intermediate-risk, IR=10-2 and 10-4, standard-risk, SR=10-4) correlated with d15 BM-MRD based stratification proposed for BM protocols (HR=10-1, IR=10-1 and 10-3, SR=10-3). In contrast, 15 BM-MRD was associated with lower relapse (5% of patients) than BM alone. However, a larger study would be needed to assess if PB-MRD could also improve the identification of HR patients, if combined with BM testing. No other treatment time point was predictive of outcome regarding PB, except for a trend at day 8 (p=0.057). Conclusions. PB-MRD at d15 identified a large group of pa-
identified age over 35 years and time to relapse <18 months as significant negative prognostic factors. For patients not transplanted in CR1, treatment with allogeneic SCT in CR2 was favoured compared to CR2 achievement without subsequent transplantation. Unexpectedly, sole bone marrow relapse was a negative factor compared to extramedullary +/- bone marrow relapse. Eleven patients, all under 35 years at diagnosis, are still alive at median 63 months (46-90) after relapse. Six patients received allogeneic and one autologous SCT. However, in older and most of the early relapsing patients, outcome was poor. Prevention of relapses is paramount, and new salvage treatments are urgently needed. piotr.kozlowski@orebroll.se

**0556**

**ACUTE LYMPHOBLASTIC LEUKEMIA IN INFANTS TREATED BY STANDARD CHEMOTHERAPY ALONE OR IN COMBINATION WITH ATRA**

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**Background.** Infants’ ALL still remain a disease with poor outcome due to the early recurrence and treatment-related mortality. Recently developed ATRA containing MLL-Baby protocol–ATRA(+) treatment approach is intended to overcome high early relapses rate without additional treatment toxicity. **Aims.** To perform comparative analysis of treatment results in patients from ATRA(+) group and patients treated by standard chemotherapy alone–ATRA(-) treatment approach. **Methods.** From July 2003 till November 2010 ninety-nine infants younger 365 days with ALL were ALL were non-randomly allocated either to ATRA(+) schedule-66 pts. or to ATRA(-) schedule-standard chemotherapy, mainly ALL-MB protocol, - 33 pts. Treatment approach was chosen under the decision of treating clinics. Both ATRA(+) and ATRA(-) groups of pts. were similar by initial characteristics: median age 6 (range 1-11) and 6 (range 0-11) months (p=0.99); sex-m/f ratio 12/21 and 24/42 (p=0.82); WBC - 96 (range 0.7-940) and 70 (range 1.6-2058) micromol/L (p=0.99); initial CNS involvement: 4 and 14 pts. (p=0.4), immunophenotype: BCPI - in 30.8% pts. and 26.9% pts. (p=0.23); biphenotype: 0 and 2.5% pts. (p=0.63), immunophenotype: BCP - in 34.6% pts. and 29.2% pts. (p=0.8), BCP - 26.9% pts. and 13.8% pts. (p=0.23); bipheno type: 0 and 1.5% pts. (p=0.63), AUL: 0 and 1.5% pts. (p=0.63); TII - 3.8% pts. and 0 (p=0.63); TIV - 4.3% pts. and 1.5% pts. (p=0.63) respectively. MLL rearrangements were detected in 45 (68.2%) among 66 pts. allocated to ATRA(+) and in 15 (53.6%) among 28 examined pts. allocated to ATRA(-) regimen. More than half of MLL-positive pts. had t(4;11): 23 out of 45 pts. from ATRA(+) group and 8 out of 15 pts. from ATRA(-) group, respectively. **Results.** We did not observe any significant difference in induction deaths: 5 (15.1%) vs. 5(7.6%), p=0.43 in ATRA(+) and ATRA(-) groups; CR rates: 28(54.9%) out of 53 pts. and 16(57.1%) out of 29 pts. (p=0.7), remission death: 0 vs. 6 (10.1%), p=0.83 respectively; but proportion of relapses remains different: 16(57.1%) from 28 pts. in ATRA(+) group vs. 11(18.6%) out of 59 pts. in ATRA(-)group, p=0.0001. Probability of RFS - 0.74±0.06 vs. 0.57±0.09, p=0.008; cumulative incidence of relapse - 0.62±0.01 vs. 0.25±0.04, p=0.01 in ATRA(+) and ATRA(-) groups correspondingly. Univariate analysis identified the following parameters to have a significant negative impact on EFS-age younger 6 months (p=0.0001); MLL rearrangements (p=0.018) and treatment without ATRA (p=0.04). Multivariate Cox regression analysis confirmed the significant negative impact on EFS-age younger 6 months with Hazard Ratio 2.550(95%CI 1.272-5.112) and ATRA(+) treatment approach (95%CI 1.072-3.796) p=0.03. Conclusion. Our data demonstrates that ATRA based approach could be treatment of choice in infants with ALL.

**0557**

**NEW FIRST LINE CHEMOTHERAPY PROGRAM WITH LINEAGE-TARGETED MTHREXATE INSURINS IS FEASIBLE AND IMPROVES THE EARLY MINIMAL RESIDUAL DISEASE RESPONSE AND SURVIVAL IN ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background/Aims.** In acute lymphoblastic leukemia (ALL) an early reduction of minimal residual disease (MRD) <10-4 confers a significant survival advantage. Since 2008 we adopted a T-ALL regimen including methotrexate (MTX) infusions at 5 g/m2. Obtaining a high rate of early molecular remissions was a major study endpoint, to improve survival also in comparison to an historical control series. **Methods.** Program N-10 consisted of 5 standard chemotherapy blocks alternating with 3 MTX blocks at 5 g/m2 (24-h infusion), folinic acid rescue 6-hourly from h 42 to a level <0.25 micromol/L; age >55 years: MTX 1.5 g/m2). This schedule targeted an in vivo MTX concentration of ~85-70 micromol/L, in keeping with the concept of lineage-targeted MTX developed at St. Jude’s Hospital. A concurrent molecular evaluation of bone marrow MRD was performed to optimize risk stratification and related therapeutic decisions. Early treatment consisted of pre-phase (PDN/CY), cycle 1 (VCR/IDR/DEX/ASP), cycle 2 (VCR/IDR/CY/DEX/AraC/6MP) and cycle 3 (VCR/IDR/DX/1AraC, plus day +30 and +70 MRD analysis). Cycles 4 and 6 were like 2, 5 and 7 like 4 (no. 5 with ASP instead of HD-AraC), and 8 like 1. Risk classes were standard (SR: thymic CD1a+ and WBC <100; CR cycle 1 and high (HR: others). Allo-SCT was prescribed to HR patients, and to SR patients with MRD >10-4 on day +70 and/or positive at later time-points. Auto-SCT followed by MEA consolidation or HD-AraC to MRD negative SR or MRD negative patients were submitted to maintenance. **Results.** All 24 evaluable patients (median age 40 years [range 17-65], SR 10, HR 14) entered CR (100%), 23 after N-10 induction and one refractory to cycle 1 after Clofarabine/AraC. Twenty patients are alive in CR1 (85%), 4 relapsed (17%) and one died in CR after SCT. With a maximum follow-up of 3 years, 2-year overall and disease-free survival are 74%. The associated MRD response was highly favorable. Day +30 MRD was negative in 62.5% (5/8) and 38% (3/9) of evaluable SR and HR patients, and respectively, and <10-4 in 2 other patients, for a major postinduction response of 75% in SR and 44% in HR. Post-MTX day +70 MRD was negative in 80% (8/10) and 67% (6/9) of SR and HR patients, respectively, and <10-4 in 2 other patients, for a major postconsolidation response of 90% in SR and 78% in HR. MTX plasma determinations at 8-h and 24-h from start of infusion were available from 57.5 g/m2 blocks administered to 19 patients, and were generally close to stated therapeutic target (8-h, mean: 78.1 [21.6], median: 75 [44-120]; 24-h, mean: 76.9 [22.5]; median 84 [27.6-124]). Extrahematologic grade III-IV toxicity was occasional (liver: 5.4%, gastro-intestinal 8%, metabolic 2.7%) and transient in nature. **Conclusions.** Protocol N-10, introducing for the first time in adult ALL 5 g/m2 MTX infusions, was feasible and highly active in adult T-ALL. Compared to the results from a previous study (protocol N-9: 84 patients, CR 85%), day +70 MRD response and 2-year survival are being improved (MRD negative 74% vs 58%; survival 74% vs 41%, p=0.04).
CURE RATES AND TOXICITY VARY ACCORDING TO AGE < VS. > 55 YEARS IN B-ALL AND BURKITT LYMPHOMA TREATED WITH THE GERMAN CHEMOTHERAPY PLUS RITUXIMAB PROTOCOL: ITALIAN STUDY ON OVER 100 PATIENTS

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Background. Mature B-ALL (acute lymphoblastic leukemia) and Burkitt lymphoma are characterized by high cell proliferation, aggressive clinical behavior and poor prognosis unless a highly specific treatment is used. The German Multicenter Study Group for Adult ALL (GMALL) recently introduced a short intensive chemotherapy program in combination with rituximab to improve results in B-ALL and Burkitt lymphoma (Hoelzer et al, ASH 2007, abstr 518). The Northern Italy Leukemia Group (NILG) adopted the same protocol to treat >100 patients since 2002. Aims. To evaluate efficacy, toxicity and long-term results obtained with this regimen in a prospective cohort of unselected patients with B-ALL and Burkitt lymphoma. Methods. Treatment consisted of six chemotherapy courses (4 courses in stage I-II disease without mediastinal or extranodal involvement) plus Rituximab (R) 375 mg/m2 for 6-8 total doses, and local radiotherapy (mediastinal or CNS involvement, or residual tumor). Treatment plan was as follow: prednisone-cyclophosphamide prephase —> R+course A (dexamethasone, vincristine, ifosfamide, HD-methotrexate, teniposide [or etoposide], Ara-C,intrathecal therapy) —> R+course B (dexamethasone, vin-cristine, cyclophosphamide, HD-methotrexate, etoposide, HD-Ara-C) —> R+A —> R+B —> R+C. Patients aged >55 years received only courses A and B but not C and lower dose methotrexate (0.5 instead of 1.5 g/m2). Results. Between December 2002 and June 2010, 106 patients were enrolled. Fifty patients had B-ALL and 56 Burkitt lymphoma (stage III-IV 28%, bulky 47%, extranodal involvement 64%). Median age was 47 years (range 17-78), 60% were male, 31% were >55 years, 16 (15%) were HIV+, 35% had an ECOG PS > 2, and 79% an elevated LDH. Eighty-three patients (78%) achieved CR, 8 had refractory disease and 15 died early (10 by infection, 3 hemorrhage, 2 other) (Table). No statistically significant difference in CR rate was observed between HIV negative and positive patients (80% vs. 69%, p=0.3). Sixty-eight patients (64%) received the whole treatment program. At a median follow-up of 3 years, 65 patients (61%) are alive in CR1, 20 (19%) died of treatment complications (TRM) and 11 developed recurrent disease (3 BM, 1 CNS, 1 BM + CNS, 6 nodal). Projected 5-year OS and DFS were 62% and 75%, respectively, with significant differences in favor of patients aged <55 years (Table). Other clinical indicators that significantly affected OS and DFS were an elevated serum LDH (P=0.03) and an ECOG PS > 2 (P=0.003). Conclusions. The short intensive German chemotherapy/rituximab regimen was confirmed effective for the management of adult patients with B-ALL and Burkitt lymphoma up to an age of 55 years (72% projected alive at 5 years). In the older age group, the lower cure rate (57% at 5 years) was equally related to TRM (mainly caused by infection) and progressive disease.
**0560**

**CD20+ PROGNOSTIC SIGNIFICANCE IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS**

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Background. The prognostic significance of CD20 expression in Acute Lymphoblastic Leukemia (ALL) blasts is still a matter of debate in adult ALL patients. These patients’ outcome has been considered to now to be variously affected both by non homogeneous chemotherapy approaches and by different biological parameters. Aim. Aim of our study was to evaluate the prognostic impact of CD20 expression in 119 adult ALL patients (<60 yrs), diagnosed according to the FAB/WHO classification and homogeneously treated between 1996 and 2009. **Methods.** Cut-off for CD20+ expression was 20%. All patients were treated according to the 0496 Gimemia Protocol (Prednisone, Vincristine, Daunolastine, Asparaginase). Patients median age was 32 years (r 15-60). Results. The median age of patients expressing CD20 (CD20+: 54 pts) was higher than of CD20- patients (42 vs 26, p=0.039), while there were no differences regarding white blood cells, platelets, percentage of peripheral and bone marrow blasts cells, recurrent genetic abnormalities (t(9;22), t(4;11), t(11;14)). CD20+ patients showed a lower incidence of myeloid antigen expression (CD13 and/or CD33, p=0.04), but this was not confirmed in CD20+ Ph- patients. There was no correlation between Ph+ (40 pts) and CD20+ and/or CD33, p=0.04), but this was not confirmed in CD20+ Ph- patients. All patients were treated according to the 0496 Gimemia Protocol (Prednisone, Vincristine, Daunolastine, Asparaginase). Patients median age was 32 years (r 15-60). Results. The median age of patients expressing CD20 (CD20+: 54 pts) was higher than of CD20- patients (42 vs 26, p=0.039), while there were no differences regarding white blood cells, platelets, percentage of peripheral and bone marrow blasts cells, recurrent genetic abnormalities (t(9;22), t(4;11), t(11;14)). CD20+ patients showed a lower incidence of myeloid antigen expression (CD13 and/or CD33, p=0.04), but this was not confirmed in CD20+ Ph- patients. There was no correlation between Ph+ (40 pts) and CD20+ and/or CD33, p=0.04), but this was not confirmed in CD20+ Ph- patients. Taking together, our data seem to suggest that CD20+ ALL adult patients are older and have a lower expression of myeloid antigens, but these data are uncorrelated with OS and DFS. Although our patients sample was a homogeneous study cohort for age and treatment, further large case series are needed to evaluate the true prognostic impact of CD20 expression in adult ALL patients and the role of CD20 antibodies therapy.

**0561**

**EFFECT OF PREVENTIVE ANTITHROMBOTIC MEASURES ON THROMBOTIC RISK IN ADULT PATIENTS TREATED FOR ACUTE LYMPHOBLASTIC LEUKEMIA**

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Background. Treatment of acute lymphoblastic leukemia (ALL) is frequently complicated by venous thromboembolism (VTE). The reported incidence varies from 2% to 37%. The highest VTE risk arises in the first treatment weeks, while the value of preventive measures is not clear yet and standardized prevention protocols are lacking. Aim. To assess the effect of various preventive antithrombotic protocols on the VTE risk in adult patients during treatment for ALL. Methods. We studied 896 adult patients aged 16-59 years with newly diagnosed ALL treated on the same anti-leukemic protocol, containing L-asparaginase in the first induction cycle, in a Dutch-Belgian multicenter study from 1999 to 2005. VTE associated during the first induction cycle were recorded. VTE prophylaxis differed between centers (no prophylaxis, frozen plasma (FP), antithrombin (AT)). We retrospectively analyzed the various preventive antithrombotic protocols during the first ALL induction cycle to assess their effect on the risk of VTE, using available patient records. Informed consent was obtained for this analysis. Results. Of 240 patients (15.0%; 95% CI 10.5-19.5) experienced VTE during protocol (10 sagittal sinus, 20 upper limb (90% central venous catheter-related), 4 deep-vein thromboses of the leg, 2 pulmonary embolisms). In 25 patients VTE occurred during the first induction cycle. Prophylactic FP compared to no VTE prevention reduced the VTE risk in the first induction cycle by nearly three-quarters (RR 0.3; 95% CI 0.1-0.5; see Table). Since prophylactic AT was only rarely given in two centers, its effect could not be properly assessed. Low-molecular-weight heparin (LMWH) was not used as regular VTE prophylaxis during this study. Conclusions. The reduced VTE risk with prophylactic FP during adult ALL induction could be explained by induced increase of AT, in contrast with previous studies that showed a negligible benefit of FP, a skewed ratio of FP protocols between centers, or another more obscure manner of FP-induced anticoagulation. Moreover, this was a retrospective quasi-randomized (by treatment center) observation. The effect of FP or other preventive VTE measures during adult ALL treatment should be confirmed by a randomized controlled study.

**0562**

**MIXED PHENOTYPE ACUTE LEUKEMIA (MPAL) ACCORDING TO THE WHO 2008 CLASSIFICATION-REPORT OF 17 CASES**

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Background. Biphenotypic acute leukemias are rare leukemias and account less than 4% of adult leukemias, characterized by coexpression of lymphoid and myeloid markers on the same leukemic cells. The diagnostic criteria until now were based on the scoring system proposed by EGIL, that was adopted by the WHO 2001 classification. The most recent edition of the WHO classification (2008) has established and published new criteria for the diagnosis of BAI, which is now termed mixed phenotype acute leukemia (MPAL). The WHO definition of MPAL is based on the expression of strictly specific T lymphoid (cytoplasmic CD3) and myeloid (MPO) antigens, shown by either flow cytometry or cytochemistry, and/or evidence of monocytic differentiation, for B-cell lineage strong expression of CD19 together with another B-cell associated marker or, in the cases with weak CD19, the expression of at least three B-lineage markers. Aim. To describe clinical features and treatment response in 17 cases of MPAL. Methods. We studied 896 adult patients with de novo acute leukemia, selected from clinical archives (1999-2010), and described clinical features, morphology and cytochemistry according to the French-American-British (FAB) criteria, immunophenotypic characteristics (by flow cytometry and immunocytochemistry), and cytogenetics by conventional karyotypic studies and molecular analysis in 17 MPAL. Patients were treated according to the national protocols for ALL or AML. Results. The final diag of MPAL fulfilled 17/896 (1.9%) patients. There were 10 male and 7 female; median age 45 (range 18-61). Morphology was consistent with ALL (65%) and AML (35%). Immunophenotyping disclosed B/myeloid variant (11/896, 1.2%), T/myeloid (5/896, 0.6%), and rare B/T (1/896, 0.1%). VTE were reported in all patients and evidenced t(9;22) (12%), in 1pt. associated to complex karyotypic changes, complex (23.5%), aberrant (23.5%) or normal (35%)karyotypes. 12% patients had not metaphases. 5 patients received ALL therapy, 10 patients

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**Table 1. Frozen plasma protocols.**
AML therapy, two patients a combination of therapy. ALL treatment induced a response in 40% patients. AML treatment in 40% patients; one patient responded to the combination of therapy. 15 (88%) patients died, 7 (41%) of resistant/resolved disease. Two patients (12%) received high dose therapy for AML followed by BMT and PBSCT, obtained CR and surviving. Overall median survival was 13.6 months and 12% of the patients are alive (1 and 11 years). Conclusion. Our results confirm a poor prognosis of MPAL. There are no uniform criteria about type of treatment in this rare disease. Adults patients should be considered for stem cell transplantation in first remission.

Aim. Prognostic significance of minimal residual disease (MRD) in acute lymphoblastic leukemia (ALL) was shown within several treatment regimens. Majority of infants with ALL carry MLL rearrangements, so in this group MRD monitoring by detection of fusion gene transcripts (FGt) could be fast, easy and cost-effective approach. MLL-Baby treatment protocol is successfully applied for infant ALL within Russian Federation and Republic of Belarus (L. Fechina et al., 2007 #2828). In this treatment approach conventional chemotherapy is augmented by administration of all-trans retinoic acid (ATRA). Aim. To evaluate the prognostic significance of MRD detected by PCR for FGT in MLL-rearranged infant ALL, enrolled into MLL-Baby study. Methods. 25 infants with defined FGT who had at least 4 available follow-up samples were included in the current study. Median of follow-up period was 30 months (range 7-90 months). Presence of MLL rearrangements was detected by nested reverse-transcriptase PCR (RT-PCR), FISH and confirmed by long-distance inverse PCR (C. Meyer et al., 2005). MRD detection in bone marrow (BM) was performed by both real-time quantitative PCR and qualitative nested RT-PCR as previously described (N. Pal et al., 1998, J. Gabert et al., 2003). MRD-negativity was defined as absence of FGT in both assays with sensitivity 1E-05. Among 25 infants with defined FGT there were 13 FGT-positive patients, 4 MLL-MLLT1-positive patients, 3 MLL-MLLT3-positive patients and one MLL-MLLT4-positive patient. BM samples were obtained at the time of diagnostics, on day 15 of remission induction (time point 1 (TP1)), at the end of remission induction (TP2) and after each ATRA course (TP3-TP9). Event-free survival (EFS) was calculated. Informed consent was obtained in all cases. Results. According to the qualitative MRD results patients were divided into MRD-positive and MRD-negative categories. All pts were MRD-positive at TP1. At TP2 3 patients became MRD-negative. At TP3 other 5 patients converted to MRD-negativity. By TP4 18 patients were MRD-negative, while FGT was detected in 5 patients. 2 patients became MRD-negative before protocol II (at TP9), while 3 patients never achieved MRD-negativity. Retrospectively all patients were referred to 3 groups in respect of MRD status at TP3 and TP4. The MRD-low risk group included 8 patients who were MRD-neg-
tion after stop-therapy was 4.5 years (range, 1 to 12 years); the mean age at the event was 12.5 years (range, 6.9 to 22 years). Table I resumes the clinical characteristics of survivors with thyreopathy. Conclusion. In our experience, the incidence of thyroid dysfunction in long survival pediatric ALL treated with chemotherapy alone results comparable to that reported in literature after cranial or craniospinal radiotherapy. The more frequent thyroid abnormalities observed were thyroid nodules with or without thyreoiditis. The short term sesame survivors do not allow a correct evaluation of thyreopathy incidence in this group of patients. We believe that childhood leukemic survivors require lifelong surveillance after completion of chemotherapy, for an early recognition and prompt treatment of late thyreopathy.

0565

USE OF CLOFARABINE IN CHILDREN WITH RELAPSED/REFRACTORY ACUTE LEUKAEMIAS; THE LARGEST SINGLE UK CENTRE EXPERIENCE

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Acute leukaemia is the most common cause of malignancy in children less than 18 years. Advances in effective treatment options have improved outcomes in recent years such that 5-year survival rates now approach 85% for ALL and 50-60% for AML. Despite these advances, approximately 20% of children with ALL experience relapse, which remains the leading cause of treatment failure (Pui & Evans, 2006). Prognosis remains poor for children who experience relapses or are refractory to front line therapy. The safety and efficacy of the combination clofarabine/cyclophosphamide/etoposide was retrospectively in children with relapsed or refractory acute lymphoblastic leukaemia (ALL) or acute myeloid leukaemia (AML) or non-Hodgkin’s Lymphoma (T-NHL). In this single centre experience we describe 14 children (10 males, 4 females) (median age 14 years, range 8-18 years) with either relapsed/refractory ALL (n=8), AML (n = 5) or T-NHL (n =1), who received clofarabine in combination with cyclophosphamide and etoposide 7 children received doses of clofarabine 40 mg/m2, cyclophosphamide 440mg/m2 and etoposide 100mg/m2 for 5 days, (doses as per CLO218 Hijiya et al) and 7 received doses of clofarabine 40 mg/m2, cyclophosphamide 800mg/m2, etoposide 150mg/m2 for 5 days, 5 along with dexamethasone. (Doses from High Risk modification arm of MB- CUKALLR3). Informed consent was obtained from all families. Of the 8 children with underlying ALL, 7 (87.5%) achieved complete morphological remission. 1 who had bulky extra medullary disease achieved good partial response. 5 of the 8 children have received a haemopoietic stem cell transplant (HSCT) (62.5%). 2 children are currently awaiting HSCT. 1 child could not receive HSCT due to invasive fungal infection. 1 child, with T-NHL, achieved remission and received HSCT. Of the 5 children with underlying had AML, 3(60%) achieved morphological remission and received HSCT. 1 child died soon after chemotherapy due to multi-organ failure, and hence response could not be assessed. 1 child received chemotherapy/HSCT/relapse and did not respond. The most common adverse events were febrile neutropenia, mucositis and reversible liver toxicity; no case of liver veno-occlusive disease was reported. Heavily pre-treated children had more side effects. These data suggest that the clofarabine/cyclophosphamide/etoposide regimen is reasonably well tolerated and can induce clinical response in a relevant proportion of children with refractory/multiple relapsed ALL and AML. Good supportive care with prophylactic antifungal and anti-PCP agents is important. (pneumocystis carinii).

0566

PROGNOSTIC IMPACT OF CD20 EXPRESSION IN ADULTS WITH DE NOVO PRECURSOR B-CELL ACUTE LYMPHOBластIC LEUKEMIA

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Background. In the last decades, we observed an improvement in treatment outcome of adult de novo acute lymphoblastic leukaemia (ALL) brought mainly by intensive chemotherapy and increased use of allo genetic hematopoietic stem cell transplant (HSCT). The addition of tyrosine kinase inhibitor chemotherapy, for patients with Philadelphia chromosome (Ph) positive ALL, was assessed by improved outcome. However, the incidence of relapse is still high in these patients. CD20 is a cell surface marker expressed in the majority of mature B-ALL blast cells, but expressed in only 40% of precursor B-ALL blast cells. Re-}

ently, a few studies have been published suggesting a worse outcome associated with CD20 expression in adult ALL. Aim: To evaluate the prognostic impact of CD20 expression in adults diagnosed with de novo precursor B-cell ALL. Methods. From 1997 to 2009, we diagnosed 147 new cases of ALL and lymphoblastic lymphoma. Fifty-four patients met criteria of inclusion in this report (diagnosis of Burkitt-type ALL, T-cell ALL and lymphoblastic lymphoma were excluded). Statistical analysis was performed with SPSS. Results. Fifty-four patients were diagnosed with precursor B-cell ALL (57.4% males; median age of 49 years, range 14-79) and were treated in first-line with Hyper-CVAD (n=44; 7 of these achieved Imatinib for Ph-positive ALL), Linker Protocol (n=2), BF12 (n=2; younger patients presenting with complex karyotype), ALL-BFM90 (n=2; less than 21 years old) and vincristine plus dexamethasone (n=4; above 70 years old). Allogeneic HSCT was performed in 15 patients. Nineteen patients expressed CD20 at a level of at least 20%. Distribution of pretreatment characteristics such as age, gender, performance status, leucocyte count, FAB subtype, LDH, CNS involvement at diagnosis and presence of Philadelphia chromosome was similar by CD20 status. First-line therapy was equality distributed between both groups. Complete response rate was similar irrespective of CD20 status (85.5% in CD20-negative vs. 84.2% in CD20-positive). There was a higher incidence of disease recurrence in the CD20-negative group (51.4% vs. 36.9%, p=0.03) and CD20-positive patients had better overall survival (OS, 39.4 months vs. 22 months, p=0.51) and disease-free survival (DFS, 72 months vs. 26.3 months, p=0.41), but this did not reach statistical significance. The median time to relapse was 11.7 months in the CD20-negative group and 16.9 months in the CD20-positive group (p=0.7). Analyzing only the subset of patients treated with Hyper-CVAD, we obtained similar results. CD20-positive patients had better OS (92% vs. 73%, p=0.3) and DFS (33% vs. 13%, p=0.52). Conclusions. Although none of the results was statistically significant, we observed a trend towards a better outcome associated with the presence of CD20 in adults with de novo precursor B-cell ALL, even in Philadelphia positive patients. Further investigation is needed to clarify the role of CD20 as a prognostic factor in adult ALL.

0567

ASSESSMENT OF ENDOCRINOLOGIC AND CARDIOLOGIC LATE EFFECTS AMONG SURVIVORS OF CHILDHOOD LEUKEMIA

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Background. Survival rates for childhood acute leukemia have significantly improved and late effects of therapy have been important in follow-up of survivors. Aims. The objective of this study is to identify the endocrinologic and cardiologic late effects of acute leukemia patients treated in our pediatric hematology unit. Patients were treated for leukemia with BFM protocols after at least five years of diagnosis were included in the study. Endocrinologic late effects (growth failure, obesity, insulin resistance, dyslipidemia, thyroid gland disorders such as hypothryoidism, osteopenia/osteoporosis, pubertal disorders) and cardiologic late effects (cardiac toxicity, hypertension) were evaluated. The study group was evaluated with anthropometric measurements, body mass index, laboratory testing of fasting glucose, insulin, serum lipids and thyroid functions. Pubertal stage was determined by using the Tanner criteria. Bone mineral densities were measured by DEXA (dual-energy X-ray absorptiometry). Blood pressures were noted. Evaluation of cardiac systolic and diastolic functions were performed using standard M-mode echocardiography and tissue doppler imaging. Results. Of 43 acute leukemia survivors with a median age of 15 (range; 7-30 years), 25 (54%) were females and 18 (46%) were males. Five (12%) of the patients had acute myeloid leukemia and 38 (85%) of them had acute lymphoblastic leukemia. They had first line therapy for an average of eight years (range; 5-17 years, SD 3.4 years). At least one adverse event occurred in 25 (58%) of the 43 survivors, with 10 of them (23%) having multiple problems. Six (14%) of the survivors were obese and 10 (23%) of them were overweight. Subjects who were overweight or obese at the time of diagnosis and at the end of therapy were more likely to be overweight or obese at last follow-up. Overweight and obesity were more frequently determined in patients who were younger than six years of age at the time of diagnosis. Insulin resistance was observed in nine (20%) subjects. Insulin resistance was more frequently seen in
subjects who are overweight or obese and who have family history of type 2 DM. Hyperlipidemia was detected in eight (18%) of the 43 survivors. Premature telarche was detected in one (2%) subject. None of the patients had short stature. Hypothyroidism was observed in one (2%) survivor. Two (5%) survivors had osteopenia. Avascular necrosis of femur head occurred in two (5%) survivors. Cardiovascular abnormalities occurred in one (2%) of the subjects with hypertension and cardiac diastolic dysfunction. No statistically significant difference was determined for the distribution of late effects between subjects who received cranial radiotherapy or not. Conclusions. In our study at least one adverse event occurred in most of the cases. Acute leukemia survivors should be followed up for the endocrinologic and cardiologic late effects with concerning sex, age at diagnosis and contents of the therapy.

**0568**

THE PROGNOSTIC IMPACT OF METHYLATED P15 AND P73 GENES IN ADULT ACUTE LYMPHOBlastic LEUKEMIA

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Background. Aberrant methylation of promoter-associated CpG islands is an epigenetic modification of DNA that plays an important role in leukemia pathogenesis. This phenomenon is frequently observed in ALL and results in the functional inactivation of its associated genes. The aim of this study was to investigate the frequency and prognostic impact of methylated p15 and p73 genes in adult acute lymphoblastic leukemia patients. Patients and Method. The study included 51 newly diagnosed adult ALL patients who presented to the Medical Oncology Department of the National Cancer Institute - Cairo University in the period from January 2008 to May 2009. Written consent was obtained from all patients. Eligible patients were adult up to the age of 50 years with adequate organ function and performance status. Mature B phenotype cases were excluded. Risk stratification based on age, initial total leucocytes count, immunophenotyping, cytogentic and complete remission rate at 4 weeks were performed at diagnosis. The treatment plan was risk adapted, standard versus high and very high risks with more intensified treatment for the very high and high risk patients. Methylation-specific polymerase chain reaction was used to analyze methylation of the p15 and p73 genes. Results. We included 30 males and 21 females, their median age was 25 years. Precursor B phenotype was detected in 37 patients while T phenotype in 14 cases. Overall risk stratification showed 18 standard (35.3%), 27 high (52.9%) and 6 very high (11.8%) risk patients. The methylation frequencies of p15 and p73 at diagnosis were 41.2% and 27.5%, respectively. Concomitant methylation was detected in 14%. The CR rate was 80.4%. No association was encountered between CR rate and methylation of a single gene, however, concomitant methylation of p15 and p73 was associated with significant lower rate of CR compared to patients with negative methylation (57% versus 90%), p = 0.008. The median survival of the standard risk patients was significantly longer than those of the high and very high risk group (15.8 versus 7 months respectively, p = 0.03). The p15 methylation status did not affect the overall survival but the p73 methylation was associated with poorer overall survival and the difference was near significant (p = 0.059). The survival benefit was significant for patient without methylation compared to patients with methylation of p15, p73 or both genes (p = 0.0053, p = 0.0004 respectively). Distribution of NG2-positive cells in tumor cells' population presented the similar differences. NG2 was heterogeneously expressed by leukemic cells' population (range 0.00%-96.80%) also in the follow-up samples. NG2 expression was detected by immunohistology and the approach were significantly higher than number of residual cells calculated according to NG2-positivity (<0.0001). We also found no correlation between NG2 expression level and normalized copy numbers of fusion genes, measured by quantitative real-time PCR. Thus, due to leukemic population heterogeneity and significant treatment-induced downexpression NG2 cannot be applied as a single marker for MRD gating, although it could be useful for previously gated cells' population description, especially in samples with low MRD-positivity. Conclusion. Due to leukemic population heterogeneity and significant treatment-related downexpression NG2 cannot be used as a single marker for MRD detection in infants with MLL-rearranged ALL. Nevertheless NG2 could be helpful as tumor-specific marker in combination with other antigens.

**0570**

EVALUATION OF ESSENTIAL STEPS TO PROMOTE ADHERENCE TO CYTOTOXIC DRUGS IN CHILDREN WITH ACUTE LYMPHOBlastic LEUKEMIA

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Background. Drug adherence is important for successful therapy in children with Acute Lymphoblastic Leukemia (ALL). Poor adherence to cytotoxic drug can cause relapse of the disease, increase therapeutic costs and may delay its cure. Evaluation of adherence to chemotherapy and taken 6-Mercaptopurine daily oral dose. Evaluation was done through specific questionnaire to the patients or his care giver which included details about the child, his family, his illness and details about the medication given and its circumstances. Also, determination of 6-mercaptopurine (6MP) metabolites was done for all children using thin layer chromatography in red cells. Results. Forty three children with ALL were included in this study, 22 males and 21 females with age ranged from 19 months to 12 years. Non adherence was detected by 6-MP level in 32.5% of cases, -ve results obtained in 9 patients (20.9%) and very low levels (< 4.5 ng in 5 patients (11.6%), and 58.1% detected by the questionnaire. Non adherence was significantly associated with low educational level (76%), 30.7 % of the non adherent children belong to a lower socio economic class, forgetfulness is the main cause of non adherence (46.2%), followed by refusal of the child to take the medicine (23%) other causes include negligence 11.5% and drug unavailability (11.5%). Mothers were the caregivers in 95 % of our patients, all well knows about the illness of their children and the consequence of stopping 6 MP; all 95% expressed with the importance of treatment. For treatment, 6-MP was given both morning and evening although 51.1 % found the cost to come to the hospital high and 48.8% find the time spent in each visit long. Age, sex, number of family members was not significant association. Conclusion. Results suggest that non adherence is mainly in-
fluenced by the educational level of the family and the low socio-economic condition. More efforts should focus on methods of assessment and prediction of adherence to medicine especially in children with cancer and studying steps to promote such adherence, further study of this problem is needed urgently especially with the more usage of oral antineoplastic drugs.

**0571**

**THROMBOTIC COMPLICATIONS IN ADULT PATIENTS WITH ACUTE LEUKEMIA: A SINGLE CENTER EXPERIENCE IN MÉXICO. INCIDENCE, RISK FACTORS AND SURVIVAL**

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**Background.** Acute leukemias are hematopoietic malignancies that may be accompanied by abnormalities in hemostasis. Thrombotic complications are the second leading cause of death in patients with cancer, and the pathogenesis is multifactorial. The use of catheters, surgery, prolonged immobilization, the start of chemotherapy (including L-asparaginase), among others, are all risk factors involved in the incidence of thrombosis. **Objectives.** To describe clinical characteristics, frequency of thrombotic events, risk factors and survival of adults with acute leukemia treated at the Instituto Nacional de Ciencias Médicas y Nutrición, “Salvador Zubirán”, Mexico City. **Methods.** A retrospective cohort of adult patients, diagnosed with acute leukemia in a specialized center, from October 2003 to December 2009. Analysis of thrombotic events, frequencies and proportions, survival curves by Kaplan-Meier, univariate and multivariate analysis were performed to determine the risk of thrombosis. Informed consent was not required. **Results.** We analyzed 181 patients with a median age of 33 years, 44.2% were women and 55.8% were male. The most common subtype was acute lymphoid leukemia (ALL) with 45.8%, being phenotype B the most frequent with 87.95%. Fifteen cases with thrombosis (8.3%) were documented, of which 53.3% were related to the use of a catheter, followed by DVT in 26.7%, acute myocardial infarction (AMI)/ischemic stroke in 13.3% and PE in 6.7%. Doppler ultrasound was the preferred diagnostic tool in 80% of cases, and the median time to develop thrombosis was 92 days, with 33.5% of events occurring during the first 30 days of diagnosis. L-asparaginase was administered in 11% of the patients, and only in 3 cases of this group (1.6%) an episode of thrombosis was also recorded. With regard to mortality, of the 15 patients with thrombosis, 27% were alive without evidence of disease at last follow-up, and 73% had died, being disease progression (46% of cases) the most common cause of death. We did not find recurrent episodes of thrombosis, and none of the events had an impact on mortality. There were no risk factors related to thrombosis in our study. The median overall survival was 349 days (range 257 to 440 days), with a follow-up of 3.5 years. **Conclusions.** The present study confirms that the incidence of thrombosis in this Mexican adult population is comparable to that reported around the globe. However, only a third of these cases were diagnosed during the first month, contrary to what has been published in recent series. And, although catheter-related thrombosis was the most frequent event in this group, our study did not confirm that this or any other factor (age, subtype of leukemia, type of chemotherapy, platelet count or WBC at diagnosis) were associated with an increased risk of thrombosis or that the thrombosis per se could be considered a negative prognostic factor regarding overall survival.

**0572**

**PULMONARY COMPLICATIONS IN SURVIVORS OF CHILDHOOD HEMATOLOGICAL MALIGNANCIES: SINGLE CENTER EXPERIENCE**

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**Background.** Children treated for cancer face the risk of complications later in life, including pulmonary dysfunction. **Aim of study.** To evaluate the frequency and severity of pulmonary complications in survivors of childhood leukemia and lymphoma treated with chemotherapy alone or combined with radiotherapy. **Methods.** 70 childhood cancer survivors (44 males and 26 females) were enrolled in this study after parental consent. They included survivors of acute lymphoblastic leukemia (n=25), acute myeloid leukemia (n=5), Hodgkin disease (n=20) and non Hodgkin lymphoma (n=20). Their age at diagnosis varied between 1-14 years (median 4 years), and their age at evaluation varied between 3-25 years (median 10 years). Exclusion criteria included the presence of primary or secondary disease in the thorax, recent major surgery, previous thoracic surgery, known major non-neoplastic lung disease, bone marrow or stem cell transplantation. Patients having evidence of chest infection at time of evaluation were temporally excluded till at least four weeks after resolution of infection. Pulmonary complications were assessed through history taking, chest examination, high resolution computed tomography (HRCT) chest, and pulmonary function testing (PFTs). **Results.** Although most survivors had no clinical pulmonary compromise, 40% had abnormal PFTs including: (14.3%) obstructive pattern, (5.7%) restrictive pattern and (20%) mixed pattern. There was no difference in PFTs between the groups in relation to malignancy diagnosis (p=0.87). Significant pulmonary dysfunction was seen in children older than 10 years of age at evaluation (p=0.002). Duration since completion of therapy was not significantly related to PFTs in multivariate analysis. Patients treated with combined chemotherapy and radiotherapy showed higher percentage of complications (72.7%) compared to those treated with chemotherapy alone (25%) (p=0.001). Cumulative dose of Bleomycin caused significant abnormal PFTs compared to other chemotherapeutic agents (p=0.04), whereas administration of methotrexate was a significant factor related to pulmonary dysfunction (p=0.002). Only male patients who received combined therapy showed higher frequency of both restrictive and obstructive lung disease, abnormal respiratory reactance and peripheral airway disease when compared to chemotherapy only group (p=0.007, p=0.04, p=0.002, p=0.006, p=0.05 respectively). There was no significant difference in female patients. Survivors with abnormal CT chest findings (n=14) had lower FVC%, FEV1% and PEF% when compared to individuals with normal CT (p=0.001, p <0.001, p=0.001 respectively). **Conclusion.** Subclinical pulmonary function abnormalities are found in survivors of childhood hematological malignancies previously treated and off therapy. Pulmonary dysfunction is more evident with combined chemotherapy and radiotherapy. Bleomycin and methotrexate are the most incriminated chemotherapeutic agents, and males are at higher risk than females. Specific and extended follow up is warranted especially in the presence of risk factors or previously detected pulmonary problems.
Acute myeloid leukemia - Biology 2

0573

DO NATURAL KILLER CELL KILLER IMMUNOGLOBULIN-LIKE RECEPTOR GROUP GENETICS AFFECT RISK OF ACUTE MYELOID LEUKAEMIA DEVELOPMENT AND TREATMENT OUTCOME?

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Legius syndrome’s gene, SPRED1, is frequently inactivated in children acute myeloblastic leukaemias (AML)

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0574

LEGIUS SYNDROM’S GENE, SPRED1, IS FREQUENTLY INACTIVATED IN CHILDREN ACUTE MYELOBLASTIC LEUKAEMIAS (AML)

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Reference


0575

SMALL SUBPOPULATIONS OF NG2 EXPRESSING BLASTS IN THE CONTEXT OF A PANMYELOID IMMUNOPHENOTYPE PREDICT FOR INVERSION INV(16) IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA (AML)

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Background. Individualized treatment is currently developed in the context of AML therapy. As a first step into this direction, treatment stratification based on cytogenetic or molecular aberrations is implicated in many current treatment protocols. Cytogenetic or molecular testing of reciprocal translocations is still the basis of this approach, but takes at least one or two days to be assessed. Flow cytometry is a standard procedure performed in the diagnostic workup of patients with AML. Recent work has shown that flow cytometric immunophenotyping allows for rapid prediction of several important cytogenetic or molecular aberrations, like t(8;21), 11q23, or mNPM1 gene. The aim of the present study was to identify an immunophenotypic pattern predicting the presence of inv(16) in AML patients. Methods. An 8-color approach (until 2006 3-color) was performed for diagnosing AML. Therefore, a FACS-Canto II (until 2006 FACS-Calibur; BD Biosciences) was used. 1,421 AML patients treated in different multicenter studies of the SAL study group were included. The NG2 monoclonal antibody (moAB 7.1, Beckman Coulter) has been used to predict the presence of aberrations at chromosome 11q23 (MLL gene) in AML and ALL, but no association was reported for inv(16) so far. The inv(16) and/or CBFβ-MYH11 was detected in 85 patients using FISH as well as PCR. Results. As a first step, the panmyeloid antigen expression (CD13+CD33+CD117+cyMPO+CD34+), CD14, or aberrant CD2 expression was shown to reach only a moderate sensitivity or specificity: 100%/71%, 59%/92%, and 28%/98%. Second, we discovered that the consideration of a low NG2 expression (1%-19%) as a unique marker was already highly sensitive and specific.
(80%–95%) in predicting inv(16). The combination of NG2 and pan-myeloid antigen expression only increased sensitivity to 95%. Finally, an integrated antigen pattern using NG2 expression plus all the above mentioned phenotypic characteristics leads to a sensitivity and specificity of 95% and 96%, respectively. Two of the 4 false negative patients showed a complex karyotype including trisomy 8. Three of those 4 patients did not achieve a CR, were MRD positive after induction therapy, or relapsed later. Conclusion: Using the integrated phenotypic characteristics described above it was possible to predict the presence of inv(16) in AML with a high sensitivity and specificity of at least 95%. Since inv(16) is sometimes hard to assess in standard metaphase preparations and requires secondary confirmation using FISH or PCR assays, flow cytometry might provide a fast and reliable screening method for rapid risk-based treatment stratification.

0576 FREQUENCY AND PROGNOSTIC SIGNIFICANCE OF MONOSOMAL KARYOTYPE IN ACUTE MYELOID LEUKAEMIA

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Monosomy karyotype (MK), defined as 2 or more monosomies, or a single monosomy, in the presence of structural abnormalities, has recently been reported as identifying a distinct subset of acute myeloid leukemia (AML) with an extremely poor prognosis. We retrospectively evaluated all AML cases with MK diagnosed in our Department over a period of 15 years and explored potential associations with clinico-biological features and outcome. Overall, karyotypic data obtained from conventional cytogenetics analysis of unstimulated bone marrow cells were available in 54% of AML cases. In our series, MK was found at a frequency of 11.3% (62/549 cases), similar to what has been reported recently in an independent patient cohort. Ninety-two percent (57/62) of MK cases were found to have a complex karyotype. Additionally, 98% (61/62) of MK cases were assigned to the unfavorable cytogenetic risk category. The median age of MK cases was 60.5 years (range, 18–88 years). MK increased with age, being present in 5.5% of cases below the age of 30 but in 15% of those over age 60 (X2-test: P=0.022). Of 51 cases with available data, 24 (47%) concerned secondary AML, whereas the remaining 27 cases (53%) were de novo AML. At diagnosis, the median white blood cell count of MK cases was 5.25x10^9/l (range, 0.7–82) and the median LDH value 349 IU (range, 100–3360). For statistical purposes, MK cases were compared to a group of 51 AML cases with unfavorable karyotypic profile yet without MK (UWMK) who were treated with similar, “+/-7”-based regimens. Complete remission rates after induction treatment were 87% in UWMK patients and 41% concurred with MK cases. In MK cases, the median overall survival (OS) of UWMK patients was 15 months versus only 6.5 months in those with MK, with 3-year OS rates of 16% and 8%, respectively MK (P=0.003). Thus, MK defines a sizeable subset of patients with unfavorable cytogenetics, often with secondary AML and advanced age, who have a particularly poor prognosis even when compared to other cases with an unfavorable cytogenetic risk profile.

0577 COMPARISON OF PROGNOSTIC MARKERS IN ACUTE MYELOID LEUKAEMIA EXCLUDING PROMYELOCYTIC LEUKAEMIA

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In contrast to acute promyelocytic leukemia (APL), which is treated with all-trans retinoic acid (ATRA), many other AML subtypes lack a simple and effective therapy. Mononuclear cells (MNC) from peripheral blood or bone marrow of 104 AML and 6 MDS patients were subjected to methylation analysis. Informed consent from all AML and MDS patients was obtained. Besides APL, other forms of APL are classified as a distinct disease entity on the basis of different genetic and functional features. AML is an heterogeneous disease with different genetic abnormalities, clinical presentation, treatment response and outcome. The aim of this study was to identify T-cell regulation genes, which are influenced by DNA methylation. LAG3 is one of these genes, which inactivation through aberrant DNA methylation could contribute to the ability of cancer cells to escape the control of immune system (IS). Mononuclear cells (MNC) from peripheral blood or bone marrow of 104 AML and 6 MDS patients were subjected to methylation analysis. Informed consent from all AML and MDS patients was obtained. Besides APL other 23 genes involved in T-cell regulation were studied using methylation-restriction endonucleases followed by QP-PCR. Bisulfite sequencing was used to validate these results and to extend number of examined samples. Levels of LAG3 gene expression were measured by TaqMan gene expression assay. The restriction-based methylation analyses showed hypermethylation of 7 genes in AML and 6 genes in MDS patients. LAG3 is one of the most promising. Frequency of hypermethylation of this gene was as follows: 5/10 AML and 5/8 MDS patients compared with 0/4 healthy donor samples. Further we made bisulfite sequencing that confirmed the same DNA methylation frequency of LAG3 gene in other 8 AML patients. By both methods we examined DNA methylation status of LAG3 gene in altogether 18 AML patients. We also performed gene expression profiling of LAG3 on the subset of 19 AML patients. We found significant downregulation of its expression in AML patients at diagnosis. Our data suggest that hypermethylation of LAG3 could play a role during process of leukemogenesis and contribute to molecular mechanisms of escape from immunological surveillance. It is a very promising gene to further evaluate its potential prognostic impact on disease outcome.

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0579 IDENTIFICATION OF SECONDARY GENETIC CHANGES IN ACUTE PROMYELOCYTIC LEUKAEMIA

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Acute promyelocytic leukemia is characterized by the chromosomal translocation t(15;17), resulting in the formation of FMS-LARα gene.

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Animal models have shown although this fusion protein is necessary it is not sufficient for leukemia development. We generated FISH probes by sequence independent amplification for chromosomal regions which have been described as altered in APL patients. The following loci were used to analyze a series of 23 APL patient samples after obtaining the inform consent: TP53, MYC, CDKN2A, CDKN1B, RB1, RASA3, NFI, KRTERT, ERG, ABCB1 and the region between PML and telomere of chromosome 15. We detected regions which are well-known to be amplified like 8q24 or deleted like 17p13 in APL, along with others like 15q24.1-qter which may harbor novel tumor suppressor gene. Deletions were detected in CDKN2A locus (30% cases), CDKN1B (26% cases), RB1 (26% cases), F53 (17% cases), ABCB1 (43% cases), PML-TEL (22% cases) and KRTERT (30% cases). Duplication were also detected in MYC (13% cases), ERG (4%), NFI (4%), and PML-TEL(4%). Our FISH assay confirmed the accuracy by which DNA copy numbers were detected by BAC array CGH in these tumor suppressors and oncogenes loci in different studies. Taken together, these results suggest that the PML/RARA fusion gene needs several other cooperating genetic lesions to cause APL.

0580
DISCOVERY OF THERAPEUTIC TARGETS FOR COMBINATION THERAPY WITH VALPROIC ACID IN ACUTE MYELOID LEUKEMIA BY INTEGRATING SCREENS IN HUMAN AND RAT AML WITH CHEMICAL-GENETIC SCREEN IN GENORHABDITIS ELEGANS
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Valproic acid (VPA) has been introduced for relapsed advance Acute Myeloid Leukemia (AML) in clinical trials. Only a subset of patients respond to this treatment. Using the Brown Norwegian Myeloid Leukemia rat as model of progressive disease under VPA therapy, we screened the phosphoproteome for molecular targets of VPA. The preclinical rat model Brown Norwegian Myeloid Leukemia (BNML) was treated with 170 mg/kg VPA twice-daily, demonstrating significant increase in survival in comparison to controls (p = 0.004). To screen for molecular targets of VPA in this highly responsive model, phosphoproteome analysis was performed by difference gel electrophoresis (DIGE) separation and subsequent differential gel software analysis. Phosphoproteins were isolated from leukemic blasts in the spleen prior to harvesting by immobilized metal ion affinity chromatography (IMAC) and identification via Orbitrap mass-spectrometry. Seven of the phosphoproteins found to be significantly differentially expressed in VPA treated BNML rats compared to controls, were investigated for functionality. This was performed by RNAi in Bristol N2 strain of C. elegans at larval stage L1, 24 hours prior to exposure to 15 mM VPA for 72 hours. 4 of 7 gene knock-downs resulted in larval developmental arrest, defined as synthetic lethality. To investigate whether this lethality was a result from apoptosis the CED-1::GFP transgenic reporter assay was employed to quantify germ line cell death following RNAi depletion and VPA exposure. Results showed increased numbers of apoptotic corpses by all genes examined. Knock-down of the 4 candidate genes in the transgenic cep-1::CED-1::GFP expressing C. elegans p53 ortholog, CEP-1, resulted in increased basal level of germline apoptosis independent of CEP-1. This suggests that a similar combinational treatment of AML patients might be beneficial, regardless of p53 status. Human AML cell line MOLM-13 was co-treated with VPA and a small molecule inhibitor against prospective target ACTB, cytochalasin B, to test this hypothesis. Inhibition of actin polymerization resulted in increased apoptosis and decreased proliferation when supplemented by VPA, as determined by DNA specific staining with Hoechst and WST-1 colorimetric assay respectively. Results indicate combination of such drugs may be beneficial in treatment of AML. Further, we used a chemical genetics synthetic lethal RNAi screen in Caenorhabditis elegans to a) explore the mechanisms of VPA regulated phosphoproteins, b) unravel why a subset of primary AML cells proliferate after treatment with VPA, c) find novel interactors of VPA by exploring chromatin associated genes, and d) find new targets for combination treatment with VPA. Indeed, we were able to discover novel genes, which increased the effect of VPA in vivo, in all screens. Interestingly, we identified novel genes involved in suppression of VPA amongst the chromatin associated genes. Especially the enhancer of VPA SERBP1 (phosphoprotein screen), and suppressor UTX (chromatin gene screen) were of interest. UTX is shown to be somatically mutated in several cancers. We conclude that novel therapeutic targets can be targeted to increase the efficacy of VPA in AML.

0581
THE PRESENCE OF FLT3-ITD AND HIGH BAALC EXPRESSION ARE INDEPENDENT PROGNOSTIC MARKERS FOR POOR OUTCOME IN PEDIATRIC AML - A MOLECULAR CHARACTERIZATION OF PATIENTS TREATED WITHIN THE NOPHO PROTOCOLS

Baalc has been identified as possible prognostic markers, including the mutation status of theFLT3, NPM1, CEBPA and WT1 genes as well as gene expression levels of ERG, MN1 and BAALC. The prognostic significance of mutations in FLT3, NPM1, CEBPA and WT1 has been thoroughly studied in adult AML but there are also many studies performed on childhood AML. However, gene expression of ERG, MN1 and BAALC and their prognostic relevance in pediatric AML have to our knowledge not been studied. Furthermore, most studies have not investigated all
these genetic markers in relation to each other and most studies have only focused on normal karyotype AML (CN-AML). Aim: To perform a thorough examination of FLT3, NPM1, BAALC, FLT3 and WT1 gene expression as prognostic markers in pediatric AML, all treated within the same NOPHO protocols, and compare their prognostic strength in relation to each other. Method. The presence of FLT3, NPM1, CEBPA and WT1 gene mutations and gene expression levels of ERG, MN1, BAALC, FLT3 and WT1 were investigated in 213 pediatric AML samples collected at diagnosis. All patients were enrolled in the NOPHO 1993 or NOPHO 2004 protocols. Results. The following results were obtained. 1) FLT3-ITD, NPM1, CEBPA and WT1 mutations were most common in CN-AML pediatric AML and they commonly co-existed. 2) The presence of the absence of NPM1 mutation was associated with significantly shorter event free survival (EFS), see K-M curve. 3) The presence of an NPM1 mutation in the absence of an FLT3-ITD correlated with improved EFS (see K-M curve) and the presence of an NPM1 mutation was associated with a better overall survival (OS) in patients with normal karyotype (p=0.058). 4) No significant correlation with survival was found for FLT3-ITD, CEBPA or WT1 gene mutations. 5) Gene expression levels of ERG, MN1 and BAALC displayed a strong positive correlation with each other. 6) High levels of ERG and BAALC transcripts at diagnosis were associated with a significant shorter EFS (ERG p<0.002 and BAALC see graph). 7) No significant correlation with survival was found for FLT3, FLT3 or WT1 gene expression. 8) In multivariate analysis, the presence of FLT3-ITD and high BAALC gene expression were independent markers for lower EFS. Conclusions. We can therefore conclude that analyzing the mutational status of FLT3 and NPM1 at diagnosis is important for correct prognostic stratification of pediatric AML patients and that determining the ERG and BAALC gene expression levels can add valuable information.

**0582**

**ROLE OF SERINE339 IN CXCR4-MEDIATED MIGRATION, HOMING AND ENGRAFTMENT OF LEUKEMIC CELLS**

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Background. The CXCR4 chemokine receptor is a major regulator of cell migration, and its overexpression has been associated with a poor prognosis notably in acute myeloid leukemia (AML). We have recently shown that the Fli1 serine/threonine kinase mediates phosphorylation of the serine residue 339 in the intracellular C-terminal tail of the receptor that interferes with CXCR4 receptor recycling and signaling. Aim. To study the role of Ser339 phosphorylation in CXCR4 mediated homing and migration, we stably expressed wildtype CXCR4 (WT) or CXCR4 mutants that abrogate phosphorylation (S339A) or imitate constitutive phosphorylation (S339E) in Kasumi-1 human AML cells lacking endogenous CXCR4 expression. Methods. The impact of these mutations on CXCR4 function was studied by measuring receptor internalization/recycling and downstream signals by cellular imaging, flow cytometry and Transwell migration assay. Results. In the presence of the ligand (CXCL12), both the normal and mutated receptors were internalized. Recycling of the receptors was observed for all variants with a slight but significant increased recycling capacity of the S339E variant. Expression of both CXCR4 mutants resulted in enhanced ERK activation when compared to the WT receptor. Intracellular calcium efflux upon ligand binding was markedly affected by the mutations: expression of CXCR4-S339A resulted in an increased efflux whereas expression of CXCR4-S339E was associated with decreased efflux when compared to WT CXCR4. Functionally, expression of WT or mutant CXCR4 was able to restore migration capacity towards CXCL12. Expression of the mutant receptors resulted in increased chemotaxis associated with increased chemokinesis (random migration capacity). Transplantation experiments in NOD-SCID mice allowed addressing the role of Ser339 in homing and engraftment. In contrast to mock-transduced cells, Kasumi-1 cells expressing WT CXCR4 showed efficient bone marrow homing and engraftment measured 24 hours and 7 days after transplantation. Interestingly, cells expressing the CXCR4-S339A or CXCR4-S339E mutant showed a significant impaired homing and engraftment capacity. Further, long-term expansion (measured 7 weeks post transplant) of grafted cells from the epididymal area to the diaphysis of the long bones was observed for all variants, suggesting that CXCR4-S339 may play a prominent role during the process of homing and engraftment in the bone marrow rather than during the process of the leukemic cells. Homing and engraftment was mostly directed to the bone marrow with only sporadic presence of cells in other CXCL12 expressing organs such as the spleen, the liver, or the meningeal space. Interestingly, in vitro adhesion and detachment assays revealed that cells expressing the CXCR4 mutants showed an increased adhesion capacity that correlated with their chemotactic potential. In addition, they displayed significantly reduced detachment properties when compared to cells expressing CXCR4-WT supporting the reduced homing capacity observed in vivo. Conclusions. Our data suggest that Ser339 phosphorylation is likely to act as an important fine-tuning mechanism for CXCR4-mediated adhesion, homing and migration of leukemic cells. Targeted interference with phosphorylation of Ser339 may therefore constitute a novel strategy to therapeutically control CXCR4 functions in normal and malignant cells.

**0583**

**SEQUENTIAL GENOMIC PROFILING IDENTIFIES POTENTIAL DRIVER ABERRATIONS IN ACUTE MYELOID LEUKEMIA FOLLOWING MYELODYSPLASTIC SYNDROME**

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Background. Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis leading to peripheral cytopenia. MDS patients exhibit an increased risk of progression towards secondary acute myeloid leukemia (sAML) with poor prognosis. The mechanisms underlying transformation from MDS to sAML are largely unknown. Aim. To identify genetic lesions that are associated with leukemogenesis we performed sequential genome-wide human single-nucleotide polymorphism (SNP) array 6.0 profiling in 32 MDS [WHO categories: RA (n=3), RCMD (n=10), RAEB-1 (n=5), RAEB-2 (n=2), CMML-2 (n=1), not classified (n=5)] and the corresponding sAML samples. Results. In total, 22 (69%) patients acquired additional genomic aberrations (copy number alterations [CNA] and/or uniparental disomies [UPD]) at diagnosis of sAML as compared to the corresponding MDS sample. The most frequent acquired numerical aberration was trisomy 8 (n=6) followed by monosomy 7 (n=3); recurrent submicroscopic losses were identified at 17q (n=13) including RUNX1, and at 21q22.12 (n=2) including RNUX1; of note, biallelic 17q11.2 and 21q21.22 losses were detected in one sAML each. Acquired UPD were detected in 6 (19%) cases affecting the following chromosomal regions: 1p (n=1), 11q (n=1), 13q (n=1), 20q (n=1), and 21q (n=2). Paired sequencing of candidate genes in 1p and 15q revealed RPLD related homozgyous mutation patterns for pre-existing heterozygous NRAS (p.G12S; chromosomal band 1p13.2) and FLT3 (internal tandem duplication; 13q12.2) mutations in the corresponding sAML cases. Paired sequencing analysis of candidate genes known to be mutated in AML (NPM1, TP53) revealed that in both NPM1 mutated sAML cases the mutation was already present at the time of diagnosis (RCMD each) diagnosis suggesting NPM1 mutations to be an early event in malignant myeloid transformation. In contrast, we also observed the acquisition of novel mutations during transformation. For example, a case initially diagnosed as RCMD with 5q- acquired a TP53 mutation during transformation to sAML with complex karyotype. Further analyses of the syndrome suggested candidate genes with potential leukemogenic relevance, in particular those mapping to 11q (WIL, CBL) and 21q (RUNX1) are currently under-way. On the other hand, genomic profiling of one sAML case following 5-azacitidine treatment of RAEB-II identified a normal genetic status in 1q and 13q, two regions found to be gained in the corresponding MDS sample. This suggests that these aberrations are not necessarily linked to transformation, and that distinct pre-existing clones might be selected by treatment for progression to AML. Summary/Conclusions. In our study more than two thirds of MDS cases ac-

**Figure 1. Kaplan-Meier curves.**
quired additional genetic abnormalities during progression to sAML. Homozygous mutation patterns of known oncogenes, such as NRAS and FLT3, as well as complete loss of tumor suppressor genes or transcription factors, such as NF1 and RUNX1, seem to play an important role in disease progression. Novel molecular analyses like next generation sequencing will facilitate to disclose additional genomic/genetic aberrations involved in this multistep process.

0584
GENOME-WIDE ANALYSIS OF REVERSIBLE EPIGENETIC ALTERATIONS MEDIATED BY RUNX1/ETO
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The reciprocal translocation of chromosomes 8 and 21, t(8;21), results in the formation of the RUNX1/ETO fusion gene that initiates acute myeloid leukaemia by recruiting co-repressor complexes to DNA. RUNX1/ETO interferes with the function of its wild-type counterpart, RUNX1, by directly targeting RUNX1 binding sites. However, the expression of RUNX1/ETO alone does not result in leukaemia and requires secondary mutations and currently it is unclear to which extent the precise epigenotype of t(8;21) cell depends on the presence of the original tumorigenic stimulus. The main purpose of our study was to (i) identify RUNX1/ETO bound target regions, (ii) determine the chromatin structure of RUNX1/ETO target regions and (iii) most and foremost to elucidate how these features are reprogrammed by the selective removal of RUNX1/ETO. To this end, we used a combination of small interfering RNA-mediated RUNX1/ETO depletion, DNASel accessibility studies, genome-wide chromatin immunoprecipitation (ChIP-seq) and expression profiling in t(8;21) carrying cell lines and cells from patients. RUNX1/ETO was found to co-localize with RUNX1, demonstrating that the fusion protein follows the binding pattern of the wild type protein but does not function primarily by displacing it. We also demonstrate that the RUNX1 binding profile and the sequence composition of RUNX1 binding cis-regulatory elements in t(8;21) and non-t(8;21) leukaemic cells is different. Integrated analysis of gene expression profiles and the RUNX1/ETO ChIP-seq readout showed that genes containing RUNX1/ETO binding sites are mostly up regulated by RUNX1/ETO knockdown. Depletion of RUNX1/ETO resulted in decreased expression of early myeloid progenitor antigens and increased expression of genes typical for differentiated myeloid cells, such as SPI1, c-fms, GCE/Polph, BPI as well as the antiproliferative genes IGFBP7 and SLA. We also show that depletion of RUNX1/ETO leads to an increase of H3K9 Acetylation and RNA Polymerase II at RUNX1/ETO bound genes. Finally we demonstrate that the level of gene expression, DNAseI accessibility, H3K9Ac and H2AZ recruitment. However, even prolonged RUNX1/ETO depletion does not significantly influence DNA methylation, indicating that the relief of epigenetic silencing of tumour suppressor genes requires additional therapeutic strategies.

0585
CO-INHIBITION OF MDM2 AND HDAC SYNERGISTICALLY ACTIVATE P53 MEDIATED APOPTOSIS IN ACUTE MYELOID LEUKEMIA IN VITRO AND IN VIVO
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Although TP53 mutations are rare in acute myeloid leukaemia (AML), wild-type p53 function is habitually annulled through over expression of MDM2 or through various mechanisms including epigenetic silencing via histone deacetylases (HDACs). We hypothesized that binary antagonism of MDM2 and HDACs, with nutlin-3 and valproic acid would additively inhibit growth in leukemic cells expressing wild-type TP53 and induce p53-mediated apoptosis. In vitro studies with the combination demonstrated synergistic induction of apoptosis in AML cell lines and patient cells. Mechanisms included massive induction of p53, acetylated p53 and p53 target genes in comparison to either agent alone. The inhibitory effect of the combinational therapy upon proliferation was correlated to the clinical parameters of the patients, where CD34 negative patient samples demonstrated significantly better response in comparison to CD34 positive samples. To evaluate the combination in vivo, we developed an orthotopic, NOD/SCID IL2rnull xenograft model of MOLM-13 (AML, FAB M5a; wt TP53) expressing firefly luciferase. Survival analysis and bioluminescent imaging demonstrated the superior in vivo efficacy of the dual inhibition of MDM2 and HDAC in comparison to controls. Our results suggest the concomitant targeting of MDM2-p53 and HDAC inhibition, may be an effective therapeutic strategy for the treatment of AML.

0586
ACUTE MYELOID LEUKEMIA INDUCES BONE MARROW FAILURE BY INDUCING DORMANCY IN NORMAL HEMATOPOIETIC STEM CELLS
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Background. Bone marrow failure (reduced production of neutrophils, platelets and erythrocytes) is an important cause of morbidity and mortality in acute myeloid leukaemia (AML). We have previously shown that normal hematopoietic stem cells (HSCs) are relatively preserved in AML while normal progenitors and differentiated blood cells are reduced indicating a block in differentiation of HSCs (Haematologica 2010; 95, suppl.2, Abstract 0031). Aim: Understand how AML induces a block in differentiation of normal HSCs. Patients and Methods: Primary samples were obtained from patients attending St Bartholomew’s Hospital, London following informed consent. Controls were untreated patients with a normal blood count and marrow examination with a non-leukemia diagnosis. 1. Cell cycle analysis of HSCs: We have previously shown that the CD34+ compartment of some AMLs (termed CD34- negative AML) is normal and contains normal stem-progenitor cells. The cell cycle status of residual HSCs (CD34+ CD38- cells) in bone marrow from patients with CD34-negative AML and controls was examined using the Ki67 assay. In addition, the proliferation of HSCs in the bone marrow of NOD/SCID/Interleukin 2 receptor gamma chain null mice transplanted with human AML cells (or controls) was assessed using the BRDU assay. 2. In vitro co-culture assay: To investigate whether the effect of AML on HSCs is dependent on cell-cell contact or due to secretion factors from AML, we cultured AML cells and
normal HSCs (CD34+ CD38-) cells or separated by a porous membrane (0.4 micron pore size to prevent cell contact). MS-5 stromal cells were used to support the AML and HSCs. Division of the HSCs was monitored using BRDU assay. Results. 1. HSCs are inappropriate quiescent in the context of AML. Normal HSCs in bone marrow from AML patients were more quiescent compared to HSCs in the control group (Figure 1A). Normal HSCs were shown to divide less in immune-stimulated conditions than controls. 2. MS-5 stromal cells secrete a soluble factor that induces quiescence in normal HSCs. This quiescence is inappropriate given the hematopoietic stress that would be expected to induce HSC cycling. This process appears to be mediated by a soluble factor. We are currently trying to identify this factor.

**0587**

MIR-34B HYPERMETHYLATION AND CREB OVEREXPRESSION MAY IDENTIFY MDS THAT EVOLVED TO AML

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Background. The cAMP response element binding protein (CREB) has been shown to be over-expressed in acute leukemia promoting abnormal proliferation, cell cycle progression, and clonogenic potential in vitro and in vivo. CREB has been previously reported to be a direct target of the microRNA, miR-34b. Low expression of miR-34b in myeloid leukemia cell lines was demonstrated to be due to hypermethylation of its promoter. Our aim is to verify in vivo the role of miR-34b in leukemia. Methods and Results. HL-60 and K562 cell lines have been transfected with lentivirus for stable expression of MiR-34b and monitored for reduced expression of CREB. The transfected cell lines were transplanted in NOD-SCID mice and in flank tumor mice models. A decreased CREB expression was confirmed, whereas engraftment and disease progression in vivo were reduced by miR-34 expression. These results confirmed an important tumor-suppressor function for MiR-34 in AML. MiR-34 expression was monitored by RQ-PCR in a large cohort of 113 patients affected by de novo AML, and in 49 pediatric patients of myelodysplastic syndrome or myelodysplastic proliferative disorder (MDS/JMML). The distribution of MiR-34 expression in 113 AML patients at diagnosis was significantly downregulated (RQ = 0.176) with respect to healthy bone marrow CD19-CD34- sorted population (RQ = 1). MDS/JMML patients presented higher levels of MiR-34b (RQ = -5.5) compared to AML at diagnosis (RQ = 1). Methylation specific-PCR revealed 65.5% (74/113) of AML patients to be methylated at the miR-34b promoter region. Methylated AML patients had lower MiR-34 expression (RQ = 0.075), with respect to the unmethylated patients (RQ = 0.573). Moreover, CREB protein expression correlated to the methylation status of the MiR-34b promoter region in AML. By contrast, all healthy samples and the 49 MDS/JMML patients showed a significant reduction of CREB expression in comparison with MiR-34b promoter hypomethylation. These results indicated that methylation of the MiR-34b/c region is a tumor-specific phenomenon and that MiR-34b controls CREB protein expression. We examined the DNA of 25 MDS patients who evolved to AML. Results showed that the MiR-34b promoter was exclusively methylated in the onset of AML, together with a decrease of MiR-34b expression levels (RQmean-MDS = 0.41 vs RQmean-AML = 0.26). We used RNA to study changes in gene expression profiles from MDS to AML using GeneChip HG U133 Plus 2.0 on paired patient samples. Supervised analysis of gene expression profiles identified 11 differentially overexpressed CREB-target genes (PRKACB, EDF1, NRXN1, FROSC, ADAM10, RAB7L1, NRP3, ITM2C, LATS2, CD6 and HOXA7 (p < 0.001) between the MDS stage and the evolution to AML. Unsupervised hierarchical clustering analysis using these 11 genes divided the 4 pairs into two separated groups, revealing CREB over-expression and activation of the CREB pathway a negative feature of disease progression from MDS to AML. Conclusion. We consider MiR-34b promoter hypermethylation to be a critical early event for the onset of AML through the activation of the CREB pathway.

**0588**

T(6;11)(q27;q23)M5/AF6 ENHANCES RAS PATHWAY WEAKENING AF6 FUNCTION IN MYELOID CELLS

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Background. Chromosomal rearrangements involving the human MLL gene at 11q23 are associated with the development of acute leukemias, and have been related to different outcome depending on the MLL translocation partner. The t(6;11)(q27;q23) translocation is characterized by MLL/AF6 expression, known to be a bad prognostic marker in acute myeloid leukaemia (AML). AF6 is a cytoplasmic-plasmatic protein that can bind RAS through its Ras Association (RA) domain, sequestering and therefore inactivating its GTP-bound active form. The exact function of the MLL/AF6 chimera and its role in the tumorigenic mechanism is still unclear. Here we focus on unraveling the process of leukemogenesis activated by MLL/AF6 fusion gene. Methods. Immunofluorescence experiment are performed to detect the localization of AF6 and RAS in healthy primary culture or in (t6;11) cell lines such as ML-2 and SHI-1. Transfection experiment are achieved to knock down AF6 or MLL/AF6 expression. Real time PCR and western blot analysis show the extent of the knockdown and the effect on downstream targets. Pathway inhibition assays confirm the important involvement of the RAS pathway in this MLL/AF6 translocated myeloid leukemia. Results. Results show the cytoplasmic co-localization and the interaction of AF6 and RAS in healthy bone marrow cells. This binding is demonstrated to reduced RAS-GTP levels. AF6 silencing in AML cells induces an increase in the expression of RAS pathway proteins by western blot, demonstrating that AF6 is crucial in maintaining the homeostasis of RAS active form and consequenlty its targets. In MLL-leukemia, the chimera MLL-AF6 is found into nucleus promoting the sequestration of the cytoplasmic AML into the nucleus as well. RAS-GTP levels were high. The silencing of the chimera in ML-2 and SHI-1, by RNA interference, shows a change in AF6 sub-cellular localization. In fact, AF6 returns in the cytoplasm and co-localize with RAS, decreasing the availability of RAS-GTP active form. Effects on the downstream pathway, after MLL/AF6 silencing, such as increase in the phosphorylation of RAF, MEK, ERK is also evident. The implication of the RAS via in t(6;11)(q27;q23) AML cells is further confirmed by using specific RAS pathway inhibitors (PD98059 and U0126). After drugs administration ML2 and SHI1 cell lines, significantly increase cell mortality and decrease colony formation capability, at the same extent as seen after MLL/AF6 silencing. Conclusion. We assume that AF6 is a cytoplasmic protein that interacts with RAS-GTP in healthy bone marrow cells, preventing the over-activation of its downstream signalling pathway. In t(6;11)-MLL/AF6 translocated AML cells, the chimera promotes AF6 removal from the cytoplasm and its reclusion into the nucleus, thereby preventing its interaction with RAS and its normal function within the hematopoietic lineage. These results suggest a possible molecular mechanism by which MLL/AF6 acts in AML to further investigated.

**0589**

HIGH-THROUGHPUT BISULFITE AMPLIFICATION SEQUENCING OF THE 14Q32 IMPRINTED DOMAIN IDENTIFIES DNA METHYLATION SIGNATURES IN ACUTE MYELOID LEUKAEMIA

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Several studies have shown that aberrant epigenetic programming, including DNA methylation, plays a role in tumorigenesis. We have previously reported that in Acute Myeloid Leukaemia (AML) a subset of miRNAs clustered in 14q32 implicated domain show a differential expression in cytogenetically distinct subtypes of AML. The domain harbours differentially methylated regions (DMRs) that regulate the expression of several imprinted genes and several binding sites for CTCF, an enhancer blocking protein, whose binding to DNA is inhibited by methylation. Hypermethylation of CTCF binding sites prevents the activity of the protein and correlates with overexpression of regulated genes. The aim of this study is to determine whether a change of DNA methylation pattern at 14q32 may occur in AML thus affecting the expression of genes and miRNAs regulated by imprinted Bisulfite amplification sequencing with Roche 454 GS FLX Titanium has been performed to analyse the DNA methylation pattern of 7 CTCF binding sites and surrounding sequences located at 14q32. The
region spanning 8 kb and overlapping the promoter of the gene MEG3, includes also the MEG3-DMR and 2 CpG islands. Twenty-four AML patients, including 14 cases with t(15;17) and 3 cases with t(8;21) translocations, 3 cases with inversion (inv16) and 4 normal karyotype (NK) have been selected for the study. Remission samples for patients with t(15;17) and NK, 4 normal bone marrow from healthy donors and 2 non-infiltrated bone marrow from lymphoma patients, were included in the analysis as controls. High-throughput sequencing of 8 amplicons ranging from 347 bp to 492 bp generated over 600,000 reads with an average sequence depth of 615 reads per amplicon. A total of 160 CpG-dinucleotides have been analysed with an average of 20 CpGs per amplicon.Unsupervised hierarchical cluster analysis showed distinctive DNA methylation signatures associated with cytogenetically different subclasses of AML. Notably, DNA methylation in 14q32 segregates leukaemia’s diagnostic specimens from remission and normal bone marrow samples. The analysis segregates also CpGs belonging to the same amplicon, suggesting that CpG methylation is non-randomly distributed in the area. AMLs displayed hypermethylation as compared to controls, with a prominent signature of methylation over hypermethylation in 14q32 segregates leukaemia’s diagnostic specimens between normal bone marrow and remission samples. Statistical analysis with ANOVA showed that 89 CpGs, distributed in 5 regions, were differentially methylated (p-value < 0.05) among all cases. In particular, 5 CpGs were differentially methylated in patients with t(15;17) as compared to controls. This pattern is consistent with miRNAs overexpression in AML with t(15;17) previously described. A highly distinctive methylation profile was detected in a region with all 33 CpGs exceeding the significance threshold (p < 0.05), that changes in 14q32 DNA methylation profile in occur in cytogenetically distinct subclasses of AML. It is likely that alteration of DNA methylation in this region could determine miRNAs deregulation.

**0589**

**PRAIME-INDUCED INHIBITION OF RETINOIC ACID RECEPTOR SIGNALING-MEDIATED DIFFERENTIATION IN AML CAN BE OVERCOME BY HIGH-DOSE ATRA**

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**Background.** In acute myeloid leukemia (AML) without retinoic acid receptor (RAR) rearrangement the effect of all-trans retinoic acid (ATRA) is still poorly understood besides a previously discovered correlation with NPM1 mutation in cytogenetically normal AML. Recently, the leukemia-associated antigen PRAME has been shown to be a dominant repressor of retinoic acid receptor (RAR) signaling in the presence of ATRA. Aims. Within this study, we now wanted to focus our efforts on further investigating the impact of PRAME expression on the reductive differentiation of ATRA induced in AML. Methods. We used AML cell models. We found that PRAME binding to and activates myeloid-specific transcription factors C/EBPα and C/EBPβ. However, mechanisms and functional outcomes of acetylation/deacetylation of myeloid-specific transcription factors are not fully understood. In the present work we aimed to analyse whether myeloid transcription factors, C/EBPα, C/EBPβ and PU.1 can be acetylated and if SIRT1 is involved in this process. We treated two promyelocytic cell lines, NB4 and HL60, with ATRA for 2 days and studied acetylation status as well as intracellular localization of above mentioned transcription factors. We found, that all three proteins were acetylated in both cell lines. In NB4 cells, acetylated C/EBPα was localized predominantly in the nucleus and its localization was not changed after stimulation with ATRA. Acetylated PU.1 was found in both nucleus and cytoplasm and ATRA treatment led to a slight increase in cytoplasmic acetylated PU.1 protein. C/EBPβ was only weakly acetylated and localized predominantly in the cytoplasm in untreated and ATRA treated cells. In HL-60 cells, acetylated C/EBPα protein was localized in the cytoplasm and was translocated into the nucleus after treatment with ATRA. Acetylated PU.1 protein was found in both cytoplasm and nucleus and incubation with ATRA resulted in slight increase of acetylated PU.1. C/EBPβ was weakly acetylated in control and ATRA-treated cells. We also found that all three transcription factors interacted with SIRT1. In both cell lines, C/EBPα-SIRT1 as well as C/EBPβ-SIRT1 protein complexes were detected in the cytoplasm and in the nucleus and ATRA treatment led to nuclear import of these complexes. Contrary, PU.1-SIRT1 complexes were localized exclusively in the cytoplasm independent of ATRA treatment. Differences in the intracellular localization of acetylated PU.1 and C/EBPα transcription factors could be explained by the reciprocal feedback influence on C/EBPα and PU.1. Conclusions. Our data demonstrate that acetylation/deacetylation of myeloid transcription factors have to be analyzed more in the details.

**0592**

**EXPRESSION OF DIFFERENTIATION-DEFECTIVE ISOFORM IV OF G-CSFR IS NOT AFFECTED IN MYELOID CELLS OF CN PATIENTS**

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Severe congenital neutropenia (CN) is a preleukemic syndrome with a cumulative incidence to develop acute myeloid leukemia (AML) or to transform into myelodysplasia (MDS) of ca. 20%. More than 80% of CN patients who developed leukemia have acquired mutations in the gene encoding granulocyte-colony stimulating factor receptor (G-CSF/SFR). Moreover, CN patients, who required doses of G-CSF higher than 8 μg/kg/d for treatment, have a higher incidence of AML/MDS, in comparison to patients with lower G-CSF doses. Therefore, we assume that aberrant G-CSFR signaling pathway could be involved in the leukemogenic transformation in CN patients. Previously, it has been shown that elevated expression of the isoform IV of G-CSFR in standard risk AML patients who received G-CSF therapy was correlated with NPM1 mutation in cytogenetically normal AML. Re-
with increased 5-year cumulative relapse incidence, in comparison to AML patients who did not receive G-CSF. Isoform IV of the G-CSFR has the membrane proximal tail responsible for proliferative functions, but replaces the carboxy-terminal region critical for maturation with a novel sequence, ablatting its ability to drive neutrophilic differentiation. In the present study we aimed to compare the expression levels of isoform IV and isoform I in myeloid cells of CN patients, CyN patients and healthy individuals. We analysed 22 CN patients (7 with ELA2 mutations, 7 with HAX1 mutations and 8 patients with unknown genetic defects), 4 CyN patients and 10 healthy individuals. We found that in healthy individuals expression levels of isoform I of G-CSFR were more than 200 (78-264) fold higher than of isoform IV. There were no significant differences in the expression ratio of isoform I to isoform IV between CN patients (median=348.7), CyN patients (median=429.7) and healthy individuals (median=199.0). There were also no differences between CN patients with different genotypes. Only one patient developed leukemia and revealed a ratio of 175. We therefore hypothesize that the expression of Isoform IV is not involved in the underlying disorder of congenital neutropenia.

High risk myelodysplasia (MDS) and acute myeloid leukaemia (AML) with associated chromosomal changes involving chromosome 5, especially as part of a complex karyotype, rarely has a durable response to cytotoxic chemotherapy. Conversely MDS patients with an isolated deletion of the long arm of chromosome 5 (del5q) frequently have a dramatic response to immunomodulatory therapy with lenalidomide. We therefore conducted a phase 2 non randomised study between August 2009 and May 2010 to assess the safety, tolerability and efficacy of lenalidomide monotherapy, followed by lenalidomide with intensive chemotherapy in patients with primary/relapsed/refractory high risk MDS or AML with abnormalities of chromosome 5. The initial lenalidomide monotherapy consisted of 10mg daily days 1-21 of a 28 day cycle. If a complete response was achieved this was consolidated with combination ADE (8+3+5 -days) - cytarabine 100mg/m2 BD, daunorubicin 50mg/m2 OD, etoposide 100mg/m2 OD) and with same dosing of lenalidomide. If a partial response was achieved a further cycle of lenalidomide monotherapy was administered, if progressing then combination induction chemotherapy with ADE (10+3+5) and lenalidomide was administered. Informed consent was obtained from 14 patients, median age 66 (range 40-75). 4 had high risk MDS, 10 had AML. 9 had primary, 3 relapsed and 2 refractory disease. Karyotypic abnormalities consisted of del5q alone in 2 and del5q with additional abnormalities in 12. All 14 patients received the initial lenalidomide monotherapy and 9 patients received combination therapy. 1 patient had only monotherapy. 4 patients discontinued the study. The primary endpoints were early death rate (EDR), the proportion of patients recovering their platelets and surviving 42 days post combination chemotherapy and response rate. Stopping rules were set for review after 10 patients had received combination chemotherapy. 3 patients died during the initial monotherapy, an early death rate of 27%, with no patients with blasts >5% achieving a remission. 1 patient with blasts <5% achieved CR after 2 cycles and continues on maintenance therapy. Of the 9 patients who received combination therapy 4 achieved CR/CRp (44%) 2 patients achieved PR (22%), for an ORR of 66%. 2 patients successfully underwent allogeneic transplantation following achievement of remission. 15 SAE's were reported, which consisted primarily of haematological toxicity, grade 3 ALT rise (22%) and venous thrombo-embolism (11%). In view of the unacceptable EDR and lack of response to monotherapy the trial was halted for consideration of amendment and simultaneous review of data for the 9 patients who had received combination chemotherapy. There were no early deaths with combination therapy, however 7 had failed to achieve platelet recovery and the stopping rule was activated. In conclusion Lenalidomide monotherapy at a dose of 10mg daily is ineffective as induction therapy in MDS/AML patients with increased marrow blasts. Lenalidomide combined with ADE chemotherapy has predictable toxicity and has efficacy even in this particularly adverse patient cohort which warrants further investigation.
OUTCOME OF RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN PATIENTS WITH ACUTE MYELOID LEUKAEMIA (AML)

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Background. 20% to 30% of AML patients relapse after allo-SCT. For these patients there is no standard therapy. They may receive intensive salvage, donor lymphocyte infusion (DLI) or palliative chemotherapy. Recently, new agents such as lenalidomide showed promising activity. We retrospectively analyse the outcome of 51 patients from our Institution who relapsed from AML following allo SCT. Methods. We selected patients with a diagnosis of AML according to WHO criteria who received allo-SCT and relapsed between Jan 2000 and Oct 2010 from our database. Primary endpoint was response rate and duration after salvage treatment. Secondary end points were the overall survival (OS) and relapse free survival (RFS) for responsive patients. At relapse, patients received either curative (anthracycline and/or high dose cytarabine and/or gemtuzumab) or non curative salvage (low dose cytarabine and/or and/or Azacytidine oral chemotherapy or best supportive care (BSC)). OS was calculated from the date of relapse. Results. Initial patients characteristics included: median age=48 years [range: 16-69], favourable-risk (n=1; 2%) intermediate-risk (n=29; 57%) or poor-risk cytogenetics (n=18; 35%). Thirty four patients (67%) had been transplanted in complete remission (31 [61%] in CR1 and 3 [6%] in CR2), and 17 (33%) with refractory disease. The conditioning regimen was myeloablative (MAC) and reduced intensity (RIC) for 9 (18%) and 42 (82%) patients respectively. Thirty three patients (65%) received an allograft from matched related donor (MRD), 8 (16%) from matched unrelated donor (MUD), 12 (23%) from cord blood and 1 (2%) from haploidentical donor. Median time from SCT to relapse was 3.9 months [0.3-90], 29 patients (57%) relapsed <6 months after SCT. Twenty two patients (43%) received curative salvage treatment, 22 (43%) non intensive chemotherapy and 7 (14%) BSC. Among the 22 patients who received intensive salvage therapy, 16 achieved CR (72%). There were 2 (9%) toxic deaths related to high dose chemotherapy and 1 patient died from extensive chronic graft-versus-host disease after a DLI. With a median follow up at 13 months [3-33], the median RFS was 5.4 months. The overall median survival (OS) was 3.4 months. Factors influencing survival were: treatment (curative vs non curative: median OS= 6.7 vs 1.3 months, p=0.015), time between allo SCT and relapse (>6 months versus <6 months, median OS= 7.7 months versus 1.9 respectively, p=0.009). Disease characteristics (cytogenetics, WHO classification) and status before graft did not significantly impact the OS. Conclusions. Our results confirm that the prognosis of the patients who relapse after allo SCT is very poor with a median 3.4 months survival underlining the need for new therapies. Initial CR and salvage treatment significantly affect survival after relapse. In a selected group of 43% of patients who relapsed after allo SCT and received intensive chemotherapy, 72% achieved CR and survived significantly longer.

CONTINUOUS SEQUENTIAL INFUSION OF FLUDARABINE AND CYTARABINE FOR ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND LOW BONE MARROW (BM) BLAST COUNT (20-30%)

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Background. According to WHO classification, the presence in the BM of 20 % or more blast cell is required for diagnosis of AML. Notwithstanding, the same WHO panel suggested that the 20% blast threshold is not a mandate to treat the patient as having AML, in that therapeutic decisions must always be based on the clinical situation after all information is considered. Accordingly, the treatment of these patients, namely in the elderly, is controversial. Aims. To investigate in a phase II trial the efficacy and toxicity of a regimen including fludarabine (F) and ARA-C (CI-ELA) in a series of 40 untreated patients aged more than 60 years with 20-30% BM blast count. A comparison with 100 patients with higher BM blast percentage uniformly treated in the same period is also presented. Methods. F at loading dose of 10 mg/sqm over 15 min at day 0, and after three hours and half ARA-C at a loading dose of 590 mg/sqm over 8 hours were given; then, F (20 mg/sqm/cl/24 hours for a total of 72 hours) and ARA-C (1440 mg/sqm over 24 hours for a total of 96 hours) were started. G-CSF was added at day +15 at a dose of 5 microg/kg. Patients achieving CR were programmed to receive a reduced dose of CI-ELA, followed by G-CSF from day 15 to mobilize CD34+ cells and perform autologous stem cell transplantation (ASCT). Between June 2001 and October 2010, 40 out of 140 patients (28%) were found with 20-30% BM blast count. Median age was 61 (61-81). Cytogenetic analysis was successful in 38 patients (95%) and showed normal karyotype (intermediate) in 24 patients (63%), while 14 patients (37%) had different chromosomal abnormalities and were classified as unfavourable. Results. Overall, 27 patients (67%) achieved CR. There were 3 induction deaths (12%), while 8 patients (20%) were refractory to induction treatment. The median number of days to neutrophil ≥0.5x10E9/l and platelet >20x10E9/l was 19 (7-34) and 20 (9-38), respectively. Documented infections occurred in 5 cases (12%). Twenty-two patients (81% of remitters) were eligible for consolidation and monitorized for mobilization of CD34+ cells, collection being successful in 15 of them (68%). Median number of CD34+ cells/kg collected was 6.8x10E6 (2.5-40.3), median number of apheresis being 2 (1-2). Thirteen patients (32% of the whole population) received ASCT. Median disease free survival (DFS) and overall survival were 9 and 10 months, respectively. Survival at 5 years is projected to 25%. The only parameter significantly related to DFS duration was the presence of unfavorable cytogenetics. In particular, DFS was 29 months for patients with diploid karyotype as opposed to 7 months for those with adverse one (p=0.001). Finally no difference was found with patients with > 30 % BM blast count as to CR achievement and duration, toxicity and overall survival. Conclusions. CI-ELA is effective and well-tolerated in elderly patients with low blast count AML. Therapeutic results are encouraging as to CR achievement and ASCT feasibility; however best results are achievable in the subgroup of patients with diploid karyotype.
**0596**

A HYPERTENSIVE PEAK SIGNIFICANTLY PRECEDES THE OCCURRENCE OF DIFFERENTIATION SYNDROME (DS) IN APL PATIENTS TREATED WITH AIDA BASED REGIMEN


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Background. Cardiovascular manifestations observed during DS, a life threatening side effect of ATRA, include hypotension, weight gain and pericardial effusion. The observation that several APL patients develop a hypertensive peak before the occurrence of DS led us to investigate its predictive value for such complication. Patients. 51 pts (31 female, 20 male) with genetically confirmed APL were treated with the Spanish PETHEMA LP99 trial between 2004 and 2010. Median age was 40 years (range, 7-71). 2 pts (4%), 51 pts (61%) and 18 pts (35%), respectively, were Sanz's low, intermediate and high risk score. Additional cytogenetic abnormalities were observed in 39% of the pts. Median body mass index (BMI) was 24 kg/m² (range, 14-40). 43 pts achieved CR (86%). DS prophylaxis included prednisone 0.5 mg/kg from d1 to d15 in high risk pts (WBC >10 G/l). A hypertensive peak was observed 24 to 96 hours (median 36) before DS in 7 pts (45.7%) of the pts who developed this complication, of whom only one had a history of hypertension. Systolic and diastolic blood pressure values varied from 160 to 260 mmHg and 95 to 110 mmHg, respectively. 4 pts died from DS. By univariate analysis, age >51, male, weight gain >5 kg, BMI >25, serum creatinine >1.4 mg/dl, low platelet count (<50,000/mm³), previous hematotoxic chemotherapy and previous sepsis were associated with DS. A hypertensive peak was seen in 43.7% of the patients who developed DS compared to 11.7% of those without DS (P=0.011). Occurrence of a hypertensive peak was independent from the use of steroid prophylaxis (P=0.45), but was significantly associated with high BMI (P=0.005). Conclusion. Hypertensive peaks during induction treatment of APL may have a predictive value on the occurrence of DS.

**0597**

VALUE OF NPM GENE MUTATIONS IN RESPONSE ASSESSMENT AND MINIMAL RESIDUAL DISEASE EVALUATION IN DENOVO CYTOGENETICALLY NORMAL NPM+ AML

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**Background.** In normal karyotype AML patients the presence of Nuclear protein megakaryoblastic 1 (NPM1) gene mutation at diagnosis is considered a good prognostic marker and FLT3 ITD negative NPM+ patients are not anymore considered for allogeneic transplantation in first complete haematological remission (CR). For minimal residual disease (MRD) evaluation the study of NPM gene mutations is not routinely performed and WT1 gene expression is considered the standard method for MRD assessment. **Aims.** We evaluated NPM gene mutations as a possible indicator of quality of response and as marker of minimal residual disease (MRD), in comparison with WT1. **Patients and Methods.** One-hundred-fifty-eight bone marrow samples of twenty-four consecutive normal karyotype NPM+ denovo AML patients (median age 54 years) treated between May 2004 and June 2010 and achieving CR after two courses of conventional chemotherapy were studied. Follow-up analyses were censored at the time of allogeneic transplantation or at the last bone marrow evaluation. A four-color flow cytometer was used to perform immunophenotyping (IF). FLT3 ITD were performed according to the international standards of quality. NPM A and B DNA mutations and WT1 expression were studied by a quantitative Real Time PCR. **Results.** In 19 out of 22 patients in whom IF was done at response evaluation the flow cytometric analyses did not detect the clonal population observed at diagnosis (immunophenotypic CR, 86%). In 12/24 patients samples were negative for NPM mutations (molecular CR, 50%). Relapse occurred in 4 patients and was associated with persistence of NPM mutations were 5/12 (42%) and 11/12 (92%), respectively (p < 0.05). Patients with the first two consecutive NPM negative samples had a lower relapse rate compared to the other patients (3/11 (27%) vs 12/13 (92%), p = 0.01). FLT3 ITD was detected in 8 patients. Five of them relapsed (median DFS 6 months, range 1-18), 3 are still in molecular CR (median DFS 21 months, range 8-69). In the follow up study of 15 patients who achieved a molecular CR the reappearance of NPM mutations was followed by haematological relapse in 8/9 patients (89%) with a median interval of 3,5 months (range 1-6 months). In 4 of these 8 NPM+ patients WT1 expression and IF were normal at the time of NPM gene evaluation. The median interval between WT1 increase and haematological relapse was 1 month (range 0-4). The median DFS in the 9 patients who achieved two consecutive negative NPM determinations and those who did not (15 patients) were 17 months (range 6-69 months) and 7.5 months (range 0-12 months), respectively. **Conclusions.** Our preliminary analysis shows that the achievement of at least two consecutive negative NPM determinations is associated with prolonged haematological remissions. In the follow up of molecular CR patients the reappearance of NPM gene mutations is almost always followed by clinical relapse and may be considered an earlier relapse marker than WT1 increase. Monitoring NPM gene mutations at response evaluation and in the follow up might help in defining subgroups of patients with high relapse risk and therefore likely to benefit of an early allogeneic transplant.

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**0598**

ACUTE MYELOID LEUKEMIA PATIENTS WITH HIGHER BONE MARROW DENDRITIC CELL LEVELS IN COMPLETE REMISSION HAVE SUPERIOR DISEASE FREE SURVIVAL

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Background. Dendritic cells (DC) are unique lineage-negative antigen-presenting leucocytes which play a critical role in the regulation of the adaptive immune response. There is limited information on a potential association between bone marrow (BM) DC levels and minimal residual disease (MRD) status and outcome in patients with acute myeloid leukaemia (AML). **Aims.** To establish levels of DC at AML diagnosis and during follow-up and relate the findings to the presence of MRD and disease free survival (DFS). **Methods.** Fifty-six out of 62 AML patients (± 65 years; 90%) achieved complete remission (CR) following intensive chemotherapy treatment at Karolinska University Hospital 2002-2006. Immunophenotyping by four-color flow cytometry (FC) on BM was performed at diagnosis to determine DC levels and aberrant blast features for MRD follow-up. Plasmacytoid DC (pDC) and myeloid DC (mDC) were identified by lin-/HLA-DR+/CD11c+ and lin-/HLA-DR+/CD123+ or lin-/HLA-DR+/CD11c+ phenotypes, respectively and expressed as percentage of the total nucleated BM cell count. DC and MRD levels were assessed at CR (n=51) and after completion chemotherapy (n=26). A hospital control group (n=10) was used to determine reference DC levels in BM. Median follow-up time for patients alive was 78 months. **Results.** In the control group median levels of pDC and mDC were 0.10% (range 0.02-0.27%) and 0.07% (range 0.02-0.27%), respectively. At AML diagnosis (n=62), pDC (median 0.00; range 0.00-1.2%) and mDC (median 0.00; range 0.00-0.8%) could be detected in only 15 and 17 patients, respectively. In all but two patients, DC levels increased at CR with median levels of pDC 0.16% (range 0.1-1.16%) and mDC 0.09% (range 0.1-5.6%). At the end of treatment the levels had in-

Table 1.
creased further, median pDC 0.38% (range 0.02-0.84%) and median mDC 0.14% (range 0.02-0.27). Patients with higher levels (defined as above the median value) of pDC at CR and after consolidation chemotherapy had longer DFS than patients with lower values, 22.5 vs. 11.5 months. No difference in DFS was observed in relation to mDC at CR (12.5 vs. 14 months) but patients with higher mDC after completion of consolidation chemotherapy had superior DFS (40 vs. 22.5 months). The number of patients with allogeneic SCT in first CR did not differ between the groups. MRD levels above 0.01-0.1% (depending on the sensitivity of the analysis) were detected in 80% of patients at CR and 35% at the end of post consolidation treatment. There was no significant association between DC levels and presence of MRD. Among 50 patients with relapse, DC levels were measured in 21 patients and were found to be almost as low as at diagnosis; median pDC 0.05% (range 0.0-0.65%), median mDC 0.03% (range 0.0-0.37%). Summary/Conclusions. No DC were seen in BM at AML diagnosis but both pDC and mDC regenerates after CR, sometimes to much higher levels than by comparison to those in control patients. DC diminished again at relapse. Patients with higher levels of DC at CR and after completion of chemotherapy seemed to have superior DFS. There was no correlation between MRD and DC levels.

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Background. Fludarabine plus Cytarabine and Idarubicin (FLAI) was proved to be an effective and well-tolerated induction regimen for treatment of acute myeloid leukemia (AML). The trial objective was to assess the efficacy of Etoposide when used in combination with FLAI schedule. Design and Methods. We retrospectively report clinical outcome results of 101 newly diagnosed and younger than 60 years AML patients (median age 46 years, range 18-60 years) treated in a Phase III clinical trial, with FLAI induction chemotherapy, including Etoposide to the FLAI schedule. Induction consisted of Fludarabine 25mg/m2/day on days 1-5, Idarubicin 6 mg/m2/day on days 1, 3 and 3, Cytarabine 2 g/m2 infused in 4 h, daily on days 1 and 5 and Etoposide 100 mg/m2/day on days 1-5. After induction, all patients underwent consolidation with Cytarabine (2 g/sqm i.v. infusion on days 1-5) and Idarubicin (12 mg/sqm i.v. infusion on days 1, 3 and 5). All the patients shared the same strategy for intensification, that was allogeneic or autologous stem cell transplantation. After consolidation, maintenance treatment with Cytarabine was given to patients who obtained a complete remission and did not undergo autologous stem cell transplantation. More than half of the patients had abnormal karyotypes. Molecular analysis at diagnosis for the more frequent abnormalities was performed. Duration of CR and overall survival was estimated according to the Kaplan-Meier method. The CR duration was dated from start of CR to first evidence of recurrence. Results. After informed consent was obtained, the patient received a single induction course of FLAI; 73 pts obtained a CR (72.2%) and 8 pts a CRp (7.9%) for an overall response rate of 80.2%. Fifteen patients (14.9%) had resistant disease, and 5 (4.9%) died during induction. After a median follow-up of 53 months, 75 patients (76%) are in continuous CR. The median CR duration and OS were 45 and 55 months, respectively. 11 pts underwent ABMT and 44 a BMT. Relapses were more frequent in patients who were not submitted to allogeneic stem cell transplantation. Of the 55 transplanted patients, 28 (51.9%); 1 with chromosome 7 abnormality), were alive in CR after a median follow-up of 10 months (range, 2 to 45 mos) after transplantation. 14 (25.9%) relapsed (median DFS 4 months), and 13 (24%) died in CR or CRp of transplant related complications. The most common grade 3 adverse events included gastrointestinal toxicities (i.e. nausea, vomiting, mucositis and diarrhoea), liver dysfunction, and skin rash. Conclusion. The combination of etoposide to FLAI is safe and active. Further studies exploring different dosing and scheduling are warranted, particularly in patients with poor-risk AML.

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AZACYTIDINE FOR ACUTE MYELOID LEUKEMIA IN ELDERLY OR FRAIL PATIENTS: A PHASE II STUDY (SAKK 30/07)

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Introduction. Acute Myeloid Leukemia (AML) in the elderly is difficult to treat. Azacitidine has been shown to improve survival in patients (pts) with myelodysplastic syndromes. We present results of a phase II trial treating elderly or frail AML pts with azacitidine. Methods. AML pts unfit for intensive chemotherapy, with a WHO performance status ≤ 3 were eligible. Therapy consisted of azacitidine 100 mg/m2 inducted subcutaneously on 5 consecutive days every 28 days for up to 6 cycles stopping at 6 cycles if no hematological improvement (HI) was observed, stopping early in case of progression or complications, and continuing beyond 6 months for responding pts. The primary endpoint was complete CR or partial remission (PR) within 6 months. Results. Between September 2008 and January 2010. 45 eligible patients (pts) from 9 Swiss centers were accrued with a median follow-up of 11 months (95% confidence interval (C.I.) [9, 13.5]). 27 (60%) were male, median age was 74 (55-86) years and 35 (79%) had performance status 0-1. 16 pts received a median of 3 (1-14) cycles. Treatment was terminated because of disease progression in 6 patients, 4 pts died in aplasia while receiving the first induction cycle of therapy. The median hospital stay in 27 pts admitted during therapy was 16 days. Among all 45 pts, 8 (18%, 95% C.I.: 8-32%) achieved CR or PR. 2/16 patients with myelodysplastic syndromes. We present results of a phase II trial treating elderly or frail AML pts with azacitidine.

AZACYTIDINE FOR ACUTE MYELOID LEUKEMIA IN ELDERLY OR FRAIL PATIENTS: A PHASE II STUDY (SAKK 30/07)

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Purpose. To study the expression of NKP30, NKP46, DNAM and ncr cytoreceptor in NK cells of patients with Acute Myeloid Leukemia (AML) at diagnosis and their correlation with survival (OS) and relapse free survival (RFS). We decided not perform analysis of APL patients because all them are alive and in remission (PETHEMA LPA 2005 protocol). Patients. 48 patients diagnosed of AML from 2006 to 2010 were included. Year median age (21-87) and 41% of them are male. The FAB categories were: M0:8; M1: 4; M2:7; M3: 4; M6: 6; M5: 15; M6:1. Karyotype were: Good risk: 10 (22%); Intermediate: 19 (41%); High risk: 15 (31%). White blood counts were 16x10⁹/µL (1.2-339x10⁹/µL); median bone marrow blast was 55% (21%-96%); 30 patients were treated according to LMA-2007 PETHEMA protocol (induction protocol Cytarabine 200 mg/m² x7 days and Idarubicin 12mg/m² x3 days; 10 patients were treated previously to LMA-2007 with the same induction schedule, consolidation and 2 cycles of Ara C intensification (only normal Karyotypes and high-risk patients were submitted to allogeneic bone marrow transplantation). Only one patient was treated with Azacitidine. Methods. NK cells from patients with AML were sorted in FACS. Expression of NK markers such as DNAM-1, NKP 46, NKp30, NCR phenotype were performd against control of normal Karyotypes and high-risk patients were submitted to allogeneic bone marrow transplantation. Only one patient was treated with Azacitidine. Expression of DNAM-1, NCRs, NKP30 and NKP46 on NK cells in patients <65 was decreased compared with age-matched controls (p<0.04). Older patients differ only in NKP46 expression (p<0.03). It was a significant inverse correlation between DNAM-1 expression on NK cells and CD112 in AML blasts. DNAM-1 on NK cells is down regulated after co-culture with AML blasts expression DNAM-ligands. Nowadays, 14 of the 48 patients are alive. The median OS is 13,467 months. The median RFS was 50,966 months. In univariate analysis, OS was correlated

LOW-DOSE LENALIDOMIDE COUPLED WITH LOW-DOSE CYTARABINE INDUCES COMPLETE REMISSION OF ELDERLY ACUTE MYELOID LEUKEMIA PATIENTS WITH UNFAVORABLE CITOGENETICS: PRELIMINARY RESULTS OF A PHASE II STUDY

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Background. Elderly acute myeloid leukemia (AML) patients frequently fail chemotherapy. Median survival does not usually exceed few months. Recently, two groups demonstrated the efficacy of high-dose lenalidomide (50 mg/day, days 1-28). However, no reports demonstrated the efficacy of low-dose lenalidomide in combination with other drugs. Aims. We designed a phase II study to assess the antitumor efficacy of the combination regimen with low-dose lenalidomide and low-dose cytarabine in patients with AML aged ≥70 years. The primary endpoint of the study was to determine the complete response (CR) rate according to SWOG criteria. Methods. Sixteen patients (median age 76 years, range: 70-80) were consecutively enrolled in the study. Median white blood cell count at diagnosis was 7.5 x10⁹/l, whereas median haemoglobin was 9.4 g/dl and median platelet count was 44x10⁹/l. Two out of sixteen patients had a normal karyotype, whereas 14/16 presented with an intermediate or unfavourable karyotype. Twelve patients had a de novo AML, whereas 4 patients had a secondary AML (2 after MDS, 1 after CML, 1 after myelofibrosis). All patients received low-dose lenalidomide (10 mg orally, days 1-21) and low-dose cytarabine (75 mg/m² x7 days). Results. Twenty-two patients were treated. 11 (50%) achieved CR or PR. Among all 16 patients 5/16 (31%) achieved CR, 1/16 (6%) achieved PR. Median time to achievement of CR was 3 months (range: 2-13 months). 5 patients completed at least one cycle of therapy did not respond at all and rapidly died due to progressive disease. At present, 5/16 patients are alive in continuous CR, 2/16 are alive with active disease, 2/16 are too early and 8/16 died either in aplasia (5) or in progressive disease (5). Notably, all responding patients presented at diagnosis t(8;21), low blast count and interstitial deletions in chromosome 5. Conclusions. Low-dose lenalidomide has clinical activity, when coupled with low-dose cytarabine, in an extremely poor-prognosis subset of AML patients. Acknowledgements. Celgene is gratefully acknowledged for providing Lenalidomide for the patients. The study was supported in part by AIL Pesaro Onlus.
with age>65 (Long-rank test: p<0.002); expression of NKP-46 (p=0.00160), NCR phenotype (Long-rank p=0.0112); expression of DNAM-1 (p<0.0297). Multivariate analysis was performed and only age (p=0.0001) and NKP-46 (p=0.05) was correlated with OS. This results point out NKP-46 in NK populations of AML patients at diagnosis as an independent factor of OS.

### 0604

**DAY 14 BONE MARROW RESULT HAS LOW POSITIVE PREDICTIVE VALUE FOR REMISSION FAILURE IN ACUTE MYELOID LEUKAEMIA**

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**Background.** The initial objective of induction therapy for patients with acute myeloid leukemia (AML) is to achieve a complete remission (CR). The conventional measure of response following induction chemotherapy is the percentage of myeloblasts on a bone marrow biopsy (BMB) performed 28 days after chemotherapy. Previous studies have suggested persistent leukemia on a BMB at day 14 (D14-BMB) is significant, and some protocols recommend immediate further treatment when persistent myeloblasts are present on D14-BMB. **Aims.** At our centre, we routinely perform D14-BMB, with the intent to immediately administer further chemotherapy if persistent myeloblasts are found. The aims of this study are to assess our adherence to this strategy, and to assess how the results of D14-BMB impact on treatment outcome. **Methods.** This was a retrospective review of consecutive patients with a new diagnosis of AML treated at our centre between 1993 and 2007. Patients were included if they had induction chemotherapy and had D14-BMB. Through chart review, their subsequent course was determined, including any therapy prior to Day 28, and subsequent bone marrow biopsy results. **Results.** There were 129 patients who had induction therapy for AML and had a D14-BMB. Forty two (32.5%) had residual leukemia on day 14, defined by myeloblasts >4%; of these, 17 had immediate reinduction chemotherapy, 5 patients were changed to supportive care, and 20 had no further action until a repeat BMB was performed at time of peripheral blood count recovery. The CR rate on the recovery or day 28 marrow for the whole group was 67.4%. The CR rate for patients with residual leukemia on day 14 was 45.2%. For patients who had additional chemotherapy after day 14 it was 47% versus 55% for patient with persistent leukemia on day 14 who had no additional chemotherapy. The CR rate for patients who had no residual leukemia on day 14 was 82.4%. For the entire group, the results of the D14-BMB resulted in any intervention in 17% of patients. The finding of a positive D14-BMB resulted in any intervention in 52% of patients. The positive predictive value of the presence of myeloblasts >4% on the day 14 BMB, in patients receiving no further action until repeat BMB at peripheral blood count recovery, was only 45%. The negative predictive value for the finding of no residual leukemia on day 14 BMB was 78%. **Conclusions.** These data confirm that the day 14 BMB result is an important prognostic factor for remission status at day 28. However, nearly half of the patients with persistent myeloblasts on day 14 BMB had no further treatment until a repeat BMB was done around day 28, and over half of these patients were in remission on day 28. In our hands, the day 14 marrow has prognostic significance but low utility in altering treatment during the induction phase of patients with AML.

### 0605

**CLOFARABINE THERAPY IN ELDERLY AML - AN IRISH REVIEW**

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**Background.** Development of acute myeloid leukemia (AML) in elderly patients is considered a condition with a very poor prognosis. Standard treatment with low-dose cytarabine has few responses in high-risk patients and only an 18% overall response rate (ORR). Clofarabine is a second-generation purine nucleoside analogue which has activity versus AML and may produce improved responses especially in high-risk groups. **Aims.** We aim to examine the outcome of elderly patients, not fit for intensive therapy, treated with clofarabine as an initial, single agent therapy. **Methods.** This is a retrospective study of patients diagnosed with AML and treated first line with single agent clofarabine across five centers in Ireland. These patients were considered unsuitable for higher intensity treatment due to age or performance status. **Results.** A total of 24 patients were treated in 5 Irish centers between July 2007 and June 2010. 82% were males. The median age at the commencement of treatment was 74 years (range 64-80) and 79% of patients (n=19) were >70 years. Of the patients for whom analysis at diagnosis was available, 60% (9/15) had a poor risk genetic profile according to WHO classification. Patients received a median of 2 cycles of clofarabine treatment (range 1-3). 89% of patients (n=20) were treated with 20mg/m² for 5 days per cycle, the remainder received 30mg/m² for their first cycle. The overall response rate was 54% (n=13). Complete remission rate was 29% (n=7), with 8% of patients (n=2) having a complete response without count recovery. The principle adverse event was neutropenic sepsis and the 30-day mortality rate was 25%. The 6-month mortality rate was 62% and the 12-month mortality rate was 89%. The overall median time of survival was 16.5 weeks (range 1-384). In those who responded it was 39 weeks (range 6.5-384 weeks) and of non-responders was 4 weeks (range 1-20). Median survival in patients > 70 years was 17 weeks (range 1-130 weeks, mean 25.5 weeks). **Conclusion.** Clofarabine as a single agent was associated with promising activity in a poor risk group of elderly AML patients with predominantly poor risk cytogenetics with a CR/CRi of 37%. Unfortunately these responses were not durable with only 11% of patients alive at 12 months. Despite a possible improvement in response rates with new agents such as Clofarabine, the optimal post-remission strategy for these patients remains a major challenge and requires further studies.

### 0606

**THE PREDICTIVE VALUE OF HEMATOPOIETIC CELL TRANSPLANTATION COMORBIDITY INDEX FOR EARLY DEATH AND SURVIVAL IN ADULT PATIENTS WITH ACUTE MYELOID LEUKAEMIA**

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**Background.** The Hematopoietic cell transplantation comorbidity index (HCTCI) predicts non-relapse mortality and overall survival (OS) after stem cell transplantation. HCTCI score is predictive of early death and survival in elderly patients with acute myeloid leukemia (AML). **Aims.** The aim of this study was to determine the prognostic role of HCTCI for early death and OS in adult AML patients of all ages. **Methods.** In the single-center, retrospective study, we analyzed the outcome of 233 AML patients aged >18 years (excluding promyelocytic AML) treated between 1999 and 2010. Eighty three percent of patients received chemotherapy. The rest of patients (17%) died before treatment (median survival 5 days, range 3-17 days) and they make a part of early death group (36%). **Results.** The median patients age was 57.3 years, ranged 18-85. Elderly population (above 60 years) makes 46% (107Pts.) of entire group. The patient population was divided into those with HCTCI scores of 0 (low score), 1 or 2 (intermediate), or 3 and more (high score) according to original
description of the HCTCI scoring system. Forty eight per cent of patients had low HCTCI scores of 0, 24% had intermediate scores of 1-2 and 28% high scores of 3 or more. Early death rates in the three groups defined as death before specific treatment or within 30 days from time of commencing induction treatment were 30.6%, 42% and 69% respectively (Chi-Square P < 0.00001). The significant difference in early death rates was present in subgroup analysis in elderly patients (>60 years, p=0.00001) as well as in younger patients (<60 years, p<0.05). When we compared all patients in early death with other patients the significantly higher WBC number (p<0.001), and HCTCI score (p<0.001) were found. OS was a median of 7.5 months in patients with a low HCTCI score of 0, 4.5 months in those with an intermediate score of 1-2, and 0.6 months in those with a high score 3 and more (p<0.001). In subgroup analysis, HCTCI score influence survival in elderly patients (p<0.01). In patients younger than 60 years there was significantly shorter survival in high HCTCI score group (0.77 months) compared with low HCTCI score group (4.9 months) (p = 0.05327) but not in other comparisons. Summary/Conclusions. The results from this study indicated that HCTCI score is predictive of early death in adult patients with AML and overall survival in elderly patients with AML. High HCTCI score also predicts shorter survival in younger patients with AML.

0607 IMPACT OF FLUDARABINE-BASED INDUCTION THERAPY ON CLINICAL OUTCOME OF CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA (CN-AML) WITH FLT3-ITD AND/OR NPM1 MUTATION

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Background. Cytogenetically normal acute myeloid leukemia (CN-AML) defines a clinical entity with an heterogeneous outcome, as 5-year survival rates range from 24 to 42%. In this patients, accounting for about 45% of total AML cases, many important prognostic factors have been identified in the last years. Among these, mutations of FLT3 and NPM1 have been most widely studied. Fludarabine-based induction therapy displayed interesting results in AML, with high rates of complete remission (CR). However, no specific data are available about fludarabine effect on the outcome of CN-AML patients according to specific molecular signature. Aims. To assess the impact of molecular abnormalities in patients treated with fludarabine, we analyzed 137 patients treated with a fludarabine-based induction therapy, evaluated response according to internal tandem duplication (ITD) in FLT3 gene and/or nucleophosmin (NPM1) mutation. Methods. One hundred thirty seven patients with CN-AML and assessed for FLT3-ITD and NPM1 mutations were included in the study. All patients received fludarabine as part of induction course, according to the institutional protocols. Patients considered at high risk of relapse for disease characteristics at diagnosis or poor response to induction therapy were considered candidate to allogeneic stem cell transplantation (SCT); in transplantated patients, survival was censored at the time of SCT. Results. Median age of patients (73 males and 64 females) was 54 years (range: 20-79), with 61 (44.5%) aged more than 55. Hyperleukocytosis (WBC > 30 x 10^9/L) and CD34 positivity were present in 50 (38.6%) and 54 (39.9%) cases, respectively. FLT3-ITD was detected in 44/137 (32%) patients. No association was found between FLT3 status and age, FAB subtype or CD34 expression, but FLT3+ patients had significantly higher WBC count (P0.05). FLT3- patients were treated with similar antileukemic induction treatment (ADE/MAE 26 pts, and DA/MC schedule 8 pts). After obtaining informed consent, samples were taken at diagnosis and also at 3rd day (D3) from start of therapy (48h of exposure in vivo). Analysis was performed on morphological level by counting cells with morphologically recognized apoptosis on at least 1000 cells and expressed as AI (%). We evaluated bcl-2 positivity in leukemic cells by immunohistochemistry with use of anti-bcl-2 antibody and imaging kits (LSAB2 and CSA, Dako Danmark) at threshold of 20% bcl-2+ cells. Statistical analysis included parametric and nonparametric tests. Results. According to morphology of blasts, 20 patients had myeloid leukemia (3 with M1 and 17 with M2). Monocytic morphology was present in 15 patients (12 had M4 and 3 had M5 type). No dysplastic features were found. Twenty three patients achieved CR (65%) and 12 were non responders. Initial mean AI was 3.4±2.2%. There was no difference in initial AI between patients according to leukemia type (myeloid/monocyte) or prognostic karyotype groups (ANOVA & Kruskal Wallis & Mann Whitney U tests p>0.05). AI was increased to 8.1±4.7% at D3 (t-test p<0.01). We have not found differences in rise of AI concerning leukemia type or cytogenetic groups. When we analyzed increase of AI and outcome of induction therapy we have found that patients achieved CR have significantly higher therapy induced AI at D3 than nonresponders (AI 6.4±5% vs. 2.5±1.9%, t-test and Mann Whitney p<0.05). Bcl-2 negative

Figure 1. Apoptosis in bone marrow.
tive patients in CR had higher increase of AAL (6.1±1.9%) than patients with NR (1.8±1.6%). That difference was much lower in group of bel2 positive patients. Conclusion. Our results in a small cohort of patients revealed that we can also measure treatment induce apoptosis in vivo, during induction treatment of AML like in vitro assays. That increase in apoptosis have impact on treatment outcome and possibly have prognostic significance like blast clearance, but further studies are needed.

**0609**

SECONDARY HEMATOLOGICAL MALIGNANCIES AFTER TREATMENT OF NON-METASTATIC BREAST CANCER

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Background. Of early breast cancer patients improved due to modern chemotherapies in last two decades. However long term toxicities as secondary hematological malignancies due to improved survival have been a major concern. Aim. We aimed to determine the frequency of secondary hematological malignancies in non-metastatic breast cancer patients (BCP) who received adjuvant chemotherapy and radiotherapy. Method. Data of BCPs followed at Hacettepe University Institute of Oncology, Department of Medical Oncology between years 2004 to 2010 were analyzed retrospectively. Results. There were 1319 non-metastatic BCPs out of 1475 followed between years 2004 to 2010. 1183 (89.7%) patients among non-metastatic BCP's received adjuvant radiotherapy and/or neoadjuvant/adjuvant chemotherapy. 1066 (80.2%) patients received adjuvant or neo-adjuvant cytotoxic chemotherapy. Adjuvant radiotherapy was applied to 960 of non-metastatic BCP's (72.8%). 228 (17.3%) patients received only adjuvant/neoadjuvant chemotherapy and 117 (8.9%) patients received only adjuvant radiotherapy. 11 (1%) out of 1066 adjuvant/neoadjuvant chemotherapy received BCPs were also treated with granulocyte colony stimulating factors (GCSF). The frequency of secondary hematological malignancies among adjuvant or neo-adjuvant chemotherapy received BCPs was 0.56% (6/1066) and 0.59% (7/1183) among radiotherapy and/or chemotherapy treated non-metastatic BCPs. There were five AML patients, three of them were AML - FAB M3 and two of them could not be subclassified. One patient had multiple myeloma and the other had diffuse large B cell lymphoma. However the latter did not receive cytoxic chemotherapy for breast cancer. Summary/conclusions. Treatment associated secondary hematological malignancies, especially myeloid leukemias is a growing problem due to high prevalence of breast cancer and dismal outcome of secondary leukemias. Further studies are needed to determine the risk for other hematological malignancies with contributing factors; radiotherapy and novel chemotherapies agents.

**0610**

COMPARATIVE STUDY OF ACUTE MYELOID LEUKEMIA WITH INV(3)/T(3:3) AND OTHER ACUTE MYELOID LEUKEMIACS ASSOCIATED WITH ABNORMALITIES OF 3Q: A GROUP OF ENTITIES WITH THE SAME CLINICAL BEHAVIOR?

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Background. Acquired abnormalities of the long arm of chromosome 3 have been reported in approximately 3-8.5% of patients with acute myeloid leukemia (AML). However, the revised 2008 WHO classifica-
23.8%). Cytogenetic risk groups were assessed according to refined MRC criteria. Results. For all 42 pts median WBC count was 27.23x10^9/L (range 1.1-177), platelet count 62.29x10^9/L (6-207), hemoglobin level 87.83g/L (47-153), peripheral blood blast percentage 30.7% (0-98), bone marrow blasts 66.7% (22-98), LDH 1216 U/L (57-2604). DIC was registered in 30/42 (71.4%) pts. Median latency period from prior CT/Pt was 54.62 months (6-245), primary disease was active in 11/42 pts. Bone marrow blast distribution was as follows: M0 (1-14), M1 (3-4, 7), M2 (1-3, 5-6, 7), M3 (2-7, 8), M4 (2, 6, 7, 8), M5 (1-4, 6-12, 14). In 5 pts FAB could not be determined and in 2 (4.8%) pts had T/My AML. Coexpression of B and T lymphoid markers was registered in 7/39 (17.9%) and 6/39 (15.4%) pts, respectively. Cytogenetic analysis was done in 33/42 (78.6%) pts. Favorable karyotype 5/33 (15.2%), intermediate 14/33 (42.4%) and unfavorable 13/33 (42.4%). Normal karyotype was registered in 9/33 (21.4%) pts, while complex and monosomal karyotypes were registered in 8/33 (24.2%) and 9/33 (27.3%) pts, respectively. Abnormalities of chromosomes 3 and/or 7 were present in 10/33 (30.3%) pts, either alone or within complex/monosomal karyotypes. Total of 24 (57.1%) pts received standard induction CT (3+7 and variants). Haematologic complete response (CR) was registered in 10/33 (30.3%) pts, either alone or within complex/monosomal karyotypes. Total of 24 (57.1%) pts received standard induction CT (3+7 and variants). Haematologic complete response (CR) was achieved in 10 (23.8%). Early death within two weeks occurred in 8 (19%) pts. For all 42 pts median overall survival (OS) was 5.94 months (range 0-84); in 10 pts achieving CR median overall survival (OS) was 11.3 months (range 0-33 months). Among all parameters assessed only pretreatment karyotype, ECOG PS, HCT-CI and active primary disease had impact on outcome. ECOG PS, HCT-CI and activity of primary disease had impact on OS. Among all evaluated patient-related and disease-related parameters only pretreatment karyotype, ECOG PS, HCT-CI and active primary disease had impact on OS. Among all evaluated patient-related and disease-related parameters only pretreatment karyotype, ECOG PS, HCT-CI and active primary disease had impact on outcome.

**0612 CLOFARABINE IN THE TREATMENT OF ACUTE MYELOID LEUKEMIA - SINGLE CENTER EXPERIENCE**

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**Background.** Clofarabine (CLO) is a purine nucleoside antimetabolite. It demonstrated its efficacy and safety not only in acute lymphoblastic leukemia patients (pts.), but also in pts. with acute myeloid leukemia (AML). Aims. To evaluate efficacy and safety of CLO in salvage treatment of AML. Methods. A retrospective analysis of pts. with AML treated with single or CLO-based regiments. For relapsed/refractory AML we used combination with antracycline (CLO 22.5 mg/m2 once daily intravenously for 5 days plus idarubicine 9 mg/m2 intravenously for 3 days, consolidation cycle with 25% dose reduction) and for pts. with molecular relapse CLO in monotherapy (CLO 22.5 mg/m2 once daily intravenously for 5 days, consolidation cycle in the same dose). Results. The median follow-up period after initial induction 2.64 to 6.42 months. Among 42 pts. we have treated 13 pts. with CLO-based regiments (9 pts. with molecular relapse and 12 pts. with hematological relapse or refractory AML). The mean age was 49 years (range 18-67 years), 7 pts. had favorable, 10 pts. intermediate and 4 pts. adverse cytogenetic risk. In relapsed pts., observed time to molecular relapse was 3.3 months (n=9) and to hematological relapse 10.2 months (n=4). From 9 pts. with molecular relapse 6 pts. (67%) achieved complete molecular remission, 1 pts. (11%) partial molecular remission and 2 (22%) had molecular or hematological progression. From 12 pts. with refractory and relapsed AML 5 (42%) achieved complete remission (CR), 5 (25%) partial remission (PR), 1 (8%) stable disease (SD), 2 (16%) progression and 1 died before assessment. 6 pts. received 1-3 cycles of CLO with CLO-based regiments (9 pts. with molecular relapse and 12 pts. with hematological relapse or refractory AML). The mean age was 49 years (range 18-67 years), 7 pts. had favorable, 10 pts. intermediate and 4 pts. adverse cytogenetic risk. In relapsed pts., observed time to molecular relapse was 3.3 months (n=9) and to hematological relapse 10.2 months (n=4). From 9 pts. with molecular relapse 6 pts. (67%) achieved complete molecular remission, 1 pts. (11%) partial molecular remission and 2 (22%) had molecular or hematological progression. From 12 pts. with refractory and relapsed AML 5 (42%) achieved complete remission (CR), 5 (25%) partial remission (PR), 1 (8%) stable disease (SD), 2 (16%) progression and 1 died before assessment. 6 pts. received 1-3 consolidation cycles and 10 pts. underwent allogeneic hematopoietic stem cell transplantation (allo HSCT) (6 pts. in CR, 4 pts. with active disease). We observed neutropenia and thrombocytopenia gr. III-IV (according CTCAE 4.0) in 100% of CLO treated pts. Infections were the most frequent complications associated with CLO therapy - 4 pts. (44%) treated for molecular relapse and 8 pts. (66%) with relapsed or refractory AML. 5 (42%) of them had severe infection (pneumonia). The frequency of infection complications decreased after introduction of posaconazole prophylaxis (66% vs.33% respectively). Mortality in molecular relapse group and relapsed/refractory disease group was 22% and 47%, respectively. 6 months disease free survival (DFS) in molecular relapse group was 85% and overall survival (OS) 75% and in relapsed/refractory disease group 75% and 50% respectively. Summary/Conclusions. The use of clofarabine shown 78% response rate in pts. with molecular relapse AML and 67% in pts. with hematological relapse or refractory AML. The most serious complications are severe infections, which could be reduced using posaconazole prophylaxis.

**0613 VALIDATION OF BONE MARROW ASPIRATES IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA AT DAY 14 OF INDUCTION THERAPY**

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Current treatment of non-M3 acute myeloid leukemia (AML) usually includes 7+3 chemotherapy followed by a bone marrow aspiration at day 14 to the beginning of therapy. This evaluation may change decisions concerning therapy and have prognostic implication. Aims. The objectives of this study were to evaluate different modalities of D14 bone marrow aspirate (BMA) evaluation (quantitatively and qualitatively), determine the inter-observer agreement and compare the results with D14 bone marrow biopsy (BMB). We included 119 patients with AML who received 7+3 and had D14 bone marrow evaluation. The analysis was performed by two independent observers, blind to patient and remission status. The evaluation included confirmation of the diagnosis of AML and identification of residual leukemia in a quantitative (percentage of blasts) and qualitative (Likert scale) manner. The qualitative assessment of blasts was determined by stratification in 5 categories: definitely infiltrated, probably infiltrated, doubtful, probably free and definitely free. Results. The evaluation of the BMA (n=107) by both observers using a Likert scale yielded a significant agreement between observers (Kappa w=0.737, p<0.001). ROC curves were obtained correlating the BMA quantification of blasts and Likert scale by both observers with bone marrow biopsy results (n=82). The areas under the curve (AUC) were 0.924 and 0.946 for observer 1 and 0.867 and 0.870 for observer 2 for assessments of the number of blasts and Likert scale, respectively. Comparing the ROC curves between the two methods of BMA evaluation (quantitative and qualitative) by the same observer we found the differences between AUC of 0.025 observer 1 (p = 0.220) and 0.002 for observer 2 (p = 0.967). The evaluation of different cutoff points for blasts percentages in BMA showed a better sensitivity and specificity at the rate below 6% and 7% blasts for observers 1 and 2, respectively. A similar analysis for the Likert scale showed the best cutoff point as the 4th item of the scale (probably infiltrated) for both observers. Conclusion. Evaluation of bone marrow at D14 using quantitative or qualitative scale had a significant agreement between observers. Both tests were predictive of bone marrow involvement comparing with biopsy. The evaluation using a qualitative scale is easier to perform.

**0614 THE COST-EFFECTIVENESS OF A NEW DIAGNOSTIC TEST THAT IDENTIFIES NEW PROGNOSTIC SUBTYPES IN ACUTE MYELOID LEUKEMIA**

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The aim of this study is to evaluate the cost-effectiveness of the new diagnostic test, the AMLprofiler, has been developed that identifies some of these new prognostic subtypes. Aim. The aim of this study is to evaluate the cost-effectiveness of the new diagnostic test, the AMLprofiler, has been developed that identifies some of these new prognostic subtypes. Aim. The aim of this study is to evaluate the cost-effectiveness of the new diagnostic test, the AMLprofiler, has been developed that identifies some of these new prognostic subtypes. Aim. The aim of this study is to evaluate the cost-effectiveness of the new diagnostic test, the AMLprofiler, has been developed that identifies some of these new prognostic subtypes.
natives: current care and if the AMLprofiler is used at time of diagnosis. Resource use was derived from all patients diagnosed in 2008 and 2009 in two hospitals. The resource use was combined with Dutch tariffs to calculate the costs for the Netherlands. Results. The AMLprofiler identifies patients who could be treated with chemotherapy instead of a (allogeneic) stem cell transplantation (SCT). These patients would not have to be exposed to the high risks of the transplantation. Other patients were identified who would need the most intensive treatment to have some chance to be cured. These patients could be given other types of allogeneic SCT (from a matched unrelated donor or cord blood transplant) instead of autologous SCT or chemotherapy. Combining this information in the decision-analytic model showed that the use of the AMLprofiler could lead to higher 5-year overall survival and lower costs. Conclusion. The AMLprofiler has the potential to be a dominant choice over current tests as the use of the AMLprofiler could lead to higher overall survival and lower costs. The health outcome could be further improved if targeted treatments were to become available for the specific subtypes of AML.

This research was supported by the Center for Translational Molecular Medicine (BioCHIP project).

### Biology of thrombosis

0615

**LARGE PNH CLONES ARE UNCOMMON IN PATIENTS WITH INTRAABDOMINAL THROMBOSIS**

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**Background.** Paroxysmal Nocturnal Hemoglobinuria (PNH) usually presents with hemolysis or cytopenias. Thrombosis, often at unusual sites-intraabdominal, cerebral or dermal—may develop during the course of the disease. However there is a paucity of data on how many cases of PNH present primarily as thrombosis. Current guidelines recommend screening for PNH in patients with the above mentioned sites of thrombosis. We screened patients with intraabdominal thrombosis to determine the frequency of the PNH clone by flow cytometry in this population. Aim. To assess the presence of PNH clones in RBCs and granulocytes, by flow cytometry in patients with intraabdominal thrombosis. Methods. Patients with intra abdominal thrombosis-Budd Chian Syndrome (BCS), Extra Hepatic Portal Vein obstruction (EHPVO), mesenteric, iliac or renal vasculature as confirmed by imaging studies and referred for thrombophilia workup to the department of Hematology, PGIMER, Chandigarh were included in the study. Patients with recent transfusions and blood samples more than 48 hours old were excluded from analysis. EDTA peripheral blood samples were analysed by flow cytometry using CD55, CD59 on both RBCs and granulocytes and additionally CD16 on granulocytes. 10,000 events were acquired and analysed on the FACS Calibur (BD Biosciences). Patients with >5% deficient RBCs and granulocytes were labelled positive for PNH clone. Normal controls were included in each run. Results. In the 3 year period, 86 adult cases of intra abdominal thrombosis were tested. There were 50 (58%), 27 (32%), 3 cases of EHPVO, BCS and Superior mesenteric vein thrombosis respectively. Iliac vein thrombosis, isolated arterial and combined arterial and venous thrombosis was seen in a single case each. Recurrent thrombosis with involvement of the abdominal vessels at least once was seen in 3 cases. Patients with EHPVO had significant cytopenias as compared to those with BCS. Small populations of CD55 deficient RBCs were seen in 8 cases (9.3%). None of the cases showed significant CD 59 negativity on the RBCs. Whereas 3 (3%) cases had granulocytes with CD 59 deficiency, CD 55 deficiency was seen in a single case. Results for CD 16 showed presence of a clone in at least 11 out of the 52 cases tested. This was not significant when compared with normals. Summary/Conclusion. Flow cytometry with CD55 and CD 59 yielded isolated small clones with a PNH phenotype in the RBCs or granulocytes of 12 (13.9%) cases patients with intraabdominal thrombosis. No case had deficiencies on both red cells and granulocytes. CD 16 was not a useful marker for detecting PNH cells in this population. Large clones of PNH type cells, reported to occur in thrombosis in known patients of PNH on follow up, were conspicuously absent in the study population. PNH with a primary thrombotic presentation is therefore rare in cases with intraabdominal thrombosis. In a resource constraint setting, effectiveness of screening all patients with intraabdominal thrombosis for PNH therefore requires reconsideration.

0616

This abstract has been withdrawn by the authors.

0617

**NITRIC OXIDE STIMULATES AND REGULATES ERYTHROPOIETIN RECEPTOR IN ENDOTHELIAL CELLS**

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**Summary/Conclusion.** Erythropoietin (EPO) is a cytokine that stimulates erythropoiesis and also induces its own receptor (EPOR) during differentiation of erythroid progenitor cells. However, as we showed previously, EPOR is expressed in cells beyond the erythroid lineage including endothelial cells. EPOR in endothelial cells is further increased at low oxygen tension. With EPO stimulation of endothelial cells during hypoxia, there was also a corresponding increase in nitric oxide (NO) production and endothelial NO synthase (eNOS) expression. We now present evidence for NO regula-
tion of endothelial EPOR expression. We use primary human umbilical vein endothelial cells (HUVECs) and the human bone marrow microvascular endothelial cell line (THBMEC) to study effect of NO donor diethylenetriamine NONOate (DETANO) on EPOR expression. HUVECs treated with 10-50 µM of DETANO at different oxygen tension for 24 hours showed statistically significant induction of EPOR gene expression at 5% and 2% of oxygen with 25 µM of DETANO and 50 µM of DETANO at 2% oxygen. Also THBMEC cultured at 21 and 2% oxygen for 3, 24, 48 hours with 50 µM DETANO demonstrated a time and oxygen dependent induction of EPOR mRNA expression after 24 hours particularly at low oxygen tension. In THBMEC and HUVEC EPOR protein was significantly induced by DETANO at 2% oxygen. We used EPO reporter gene assay to examine if NO could regulate EPOR expression at transcriptional level. HeLa cells were transfected with different constructs containing the EPO 5’ UTR and extending upstream to about 2 kb and 200 bp from the transcription start site. DETANO treated HeLa cells, with the 2 kb promoter construct, increased luciferase activity at 2% oxygen about 50 %, and 70 % with the 200 bp construct. There was no induction of EPO promoter activity after DETANO stimulation at 21% of oxygen, suggesting that NO regulated EPOR expression at the transcriptional level in promoter region only at low oxygen tension. Different signaling pathways in THBMEC were examined after DETANO stimulation and we found that DETANO activated MAPK kinase in THBMEC after 30 min both at 21% and more at 2% oxygen. The effect of EPO on MAPK activation was not statistically significant. However, both EPO and DETANO induction of EPOR were blocked with MAPK inhibitor PD98059, suggesting direct effect of DETANO on MAPK kinase activation. These data provide a new effect of NO on EPOR expression and regulation in endothelial cells under normal and low oxygen tensions.

0618

NEW METHOD OF PROTHROMBOTIC TENDENCIES PREDICTION IN SEPSIS: SPATIAL CLOT GROWTH

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Background/Aims. Inflammation in sepsis activates blood coagulation that may lead to thromboses of the vessels of the microcirculation and consequently organ failure. So estimation of haemostasis system state is extremely important. Conventional diagnostic methods are insensitive to procoagulant changes in sepsis. Our aim was to study changes in haemostasis system state for patients with sepsis and septic shock by a method of spatial clot growth created in our laboratory and to compare the sensitivity of this new method to conventional ones.

Methods. 16 patients (age 21-60) with hematological malignancies and sepsis were enrolled in the study. All patients have been surveyed from 1st to 5th day and on 7th, 14th, 21st and 28th days from the infection beginning. The spatial clot growth is the original method of investigation of haemostasis. Its principle consists in registration of clot formation after local activation of coagulation by immobilized tissue factor by light scattering in non-stirred thin layer of platelet-free plasma containing inhibitor of contact activation. Contemporarily clotting time, thromboelastography, plasma D-dimer level assay were performed. Results. Spatial clot growth showed growing hypercoagulation in 6 patients. Plasma D-dimer levels rose after that in 5 of them. Contrarily, plasma D-dimer levels did not raise and were statistically significant lower if spatial clot growth did not show hypercoagulation. Mean values were 487 µg/l and 254 µg/l, respectively (p<0.05). Other tests did not show hypercoagulation during all time of the study. Other 10 patients had elevated plasma D-dimer level on 1st day. It gradually reduced or stayed the same. Spatial clot growth showed normalization of coagulation in survivors or growing hypocoagulation in non-survivors. Routine tests showed normal and hypocoagulation or only hypocoagulation revealed in them. Conclusions. Spatial clot growth is more sensitive to the procoagulant changes of haemostasis system state than routine tests and can predict prothrombotic tendencies in sepsis.

The study was supported by the RFBR grants 09-04-02323, 09-02-00018, 09-04-03537, 09-04-92427, 10-01-91055, and by the RF President Grant for Young Scientists MK-155.2010.4.

0619

DIVERGENT ROLE IN THROMBOTIC AND INFLAMMATORY MECHANISMS REGULATED BY THE G455A POLYMORPHISM OF THE BETA CHAIN OF FIBRINGEN IN PATIENTS WITH STABLE ANGINA

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Purpose. Patients with coronary artery disease (CAD) exhibit increased levels of thrombotic and inflammatory biomarkers including fibrinogen, interleukin 6 (IL-6) and sCD40L. However, it remains obscure, whether the genetic variability on fibrinogen beta chain modifies thrombosis and inflammation in those patients. In the present study we investigated the impact of the G455A genetic polymorphism on the aforementioned biomarkers. Methods. We genotyped 395 patients with documented CAD and 236 controls. The G455A polymorphism was determined by polymerase chain reaction (PCR) and the specific Haell restriction enzyme. Serum levels of fibrinogen were measured by the von Clauss method. Moreover, IL-6 and sCD40L levels were assessed by enzyme-linked immunosorbent assay (ELISA). Results. Genotype distribution was GG: 54.6%, GA: 36.8%, AA: 8.6% for controls and GG: 50.1%, GA: 42.0%, AA: 7.9% for CAD. Interleukin 6 levels (pg/ml) were enhanced in patients with CAD compared to controls (4.2±0.9 vs 4.1±2.76, p<0.01). Similarly, serum sCD40L levels (ng/ml) were significantly higher in CAD compared to controls (1.9±0.7 vs 1.8±0.68, p<0.001). Although no difference was observed in sCD40L across the study genotypes both in controls and in CAD (p=NS), the G455A polymorphism defined sCD40L levels (GG+GA vs AA both in CAD and controls (p=NS). Similarly, sCD40L levels (ng/ml) were significantly higher in CAD compared to controls (4.2±0.7 vs 3.7±0.97, p<0.01). Importantly, the present polymorphism affected significantly fibrinogen levels (GG+GA vs AA) not only in controls (366.6±85.8 vs 439.1±122.3, p<0.05), but also in CAD (246.0±122.7 vs 251.8±113.1, p<0.001). Conclusions. The G455A genetic polymorphism has a striking effect on fibrinogen levels both in controls and in patients with coronary artery disease. In addition, it affects partly sCD40L in the total population. Our findings suggest that the G455A polymorphism affects the thrombotic process and consequently promotes atherosclerosis, especially via its significant impact on fibrinogen.
and G2R, the father heterozygous for L210F+X43 had type I deficiency, whereas heterozygous G2R mutation had null effect in the mother. Among the asymptomatic women, three with type I deficiency had two novel (S250F+X16 and V308C+X16) and one known (C4-X) heterozygous mutations. Four with type II HBS deficiency had three known heterozygous mutations (R474H, K47C, 1.99 -two cases), in one of them associated with nonprothrombin G20210A (a.9499). In four pregnant women (one type I and three type II HBS) antithrombotic prophylaxis was tailored according to the phenotype. Conclusions. The molecular bases of AT deficiency are heterogeneous; their identification can provide data to understand AT structure-function and to give advice for antithrombotic prophylaxis tailored according to different AT deficiency subtypes.

0621
THE RELEASE OF PLATELET FACTOR 4 (PF4) IS IMPAIRED IN PATIENTS WITH ET, ESPECIALLY THOSE PRESENTING BLEEDING COMPLICATIONS

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Background. Essential thrombocytopenia (ET) is characterised by bleeding tendency, thrombotic complications and qualitative platelet defects. PF4 is synthesized in megakaryocytes and stored in platelet granules; its activation by thrombin takes place in the vicinity of vessel wall injury. That is why PF4 is the most important marker of platelet activation. According to the literature, clot formation and stability are optimal at physiological levels of PF4. Both increased and decreased PF4 levels in platelets disrupt normal clot development and may indicate impaired platelet function and impaired clot formation.

Methods. We have examined 106 ET patients and looked for correlation between these parameters and bleeding or thrombotic complications. We have also examined 106 patients with ET (80 females and 28 males, mean age 54 (23-82). The control group (CG) consisted of 20 healthy persons: 6 males and 14 females (mean age 41 (31-54)). We evaluated serum level of PF4, cholesterol and triglycerides and plasma activity of factors: I, VIII, XII, AT, protein C and S. We assessed also von Willebrand factor antigen and plasma activity of main coagulation factors in a group of 106 ET patients and looked for correlation between these parameters and bleeding or thrombotic complications.

Results. The molecular bases of AT deficiency are heterogeneous; their identification can provide data to understand AT structure-function and to give advice for antithrombotic prophylaxis tailored according to different AT deficiency subtypes.

0622
SENSITIVE APTT FOR LUPUS ANTICOAGULANT (LA) DETECTION. IS IT USEFUL AS A FIRST STEP IN A BASED-TEST ALGORITHM OF CLINICALLY SUSPECTED ANTIPHOSPHOLIPID SYNDROME (APS) DIAGNOSIS?

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Background. According to the guidelines for LA detection updated in 2009 by the ISTH (V. Fenoglio et al., JTH 2009; 9: 1737-40), testing for LA should be limited to patients with a significant probability of having APS, or unexplained prolonged aPTT. Single test is not sensitive to detect LA, being necessary to perform two different tests: Diluted Russell Viper Venom Time (dRVVT) as first choice, followed by a sensitive aPTT (low phospholipid and silica as activator). Nevertheless in some centers aPTT is the first choice for LA detection. Aim. To define a laboratory based algorithm depending on the test performed for the first step evaluation in LA detection in patients with APS. Patients and Methods. Along a period of two months, 220 patients underwent evaluation for suspected hypercoagulability status. In cases with clinical criteria of APS according to the Sapporo International Consensus Statement, the clinical files (demographic, clinical and inherited and acquired thrombophilic risk factors) of patients were reviewed. Sensitive aPTT and dRVVT were performed using an ACL TOP-3G automatized coagulometer (Instrumentation Laboratory).

Results. 220 patients were evaluated for hypercoagulable status (including LA). Positive result for LA was detected in 45 patients (19.5%) (sensitive aPTT: 9.3% and dRVVT: 25.58% (p=0.003)). In median age of 50 years (range: 5-82). Prolonged sensitive aPTT was detected in 19 of these 43 patients (44.2%), M 9 (47.4%) and F 10 (52.6%) with a median age of 52 years (range: 5-79). Clinical events were deep vein thrombosis/pulmonary embolism 14 (32.5%); cerebrovascular disease 9 (20.9%); arterial ischaemia 5 (11.6%); venous and arterial thrombosis 2 (4.6%), acetic complications 1 (2.3%); connective tissue disease 3 patients (18%); and G2R; the father heterozygous for L210F+X43 had type I deficiency, whereas heterozygous G2R mutation had null effect in the mother. Among the asymptomatic women, three with type I deficiency had two novel (S250F+X16 and V308C+X16) and one known (C4-X) heterozygous mutations. Four with type II HBS deficiency had three known heterozygous mutations (R474H, K47C, 1.99 -two cases), in one of them associated with nonprothrombin G20210A (a.9499). In four pregnant women (one type I and three type II HBS) antithrombotic prophylaxis was tailored according to the phenotype. Conclusions. The molecular bases of AT deficiency are heterogeneous; their identification can provide data to understand AT structure-function and to give advice for antithrombotic prophylaxis tailored according to different AT deficiency subtypes.

0623
ASSOCIATION BETWEEN INTERLEUKIN-6 AND CORONARY ARTERY DISEASE SEVERITY AMONG NON-ELDERLY PATIENTS

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Background. Systemic inflammation and carotid atherosclerosis markers have been used to detect patients at high risk of cardiovascular disease. We investigated the association of hs-CRP and a cytokine panel (IL-6, IL-1b, IL-8, IL-10 and TNF-a) to coronary artery disease (CAD) extent, accounting for the effect of subclinical carotid atherosclerosis. Methods. Eighty-three patients (92.8 % males, 54.5±7 year-old) had their first coronary angiogram after an acute coronary syndrome (81.9%) or first coronary angiogram (12.6%) or unstable angina (5.7%). 17 patients presented venous thrombosis and 3 patients arterial thrombosis. No prolonged sensitive aPTT was detected in absence of LA positive detection. Conclusions. 1) According to the latest updated recommendations, dRVVT must be considered as the first choice as first step in LA detection due to the possibility of misdiagnosis when sensitive aPTT is performed as first choice. 2) Combination of both tests seems to be the better choice in performing a test-based algorithm for LA detection. 3) Guidelines for LA detection and their updating in facets of test performance including preanalytical, analytical, and postanalytical issues are necessary to improve knowledge and experience.

0624
ASSOCIATION BETWEEN INTERLEUKIN-6 AND CORONARY ARTERY DISEASE SEVERITY AMONG NON-ELDERLY PATIENTS

M El-Ali, T Theodoridis, S Koulouris, K Triantafylfou, M Karantza, A Manolis, V Christopoulou- Cokkinou
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osclerosis indices. Patients in the highest IL-6 concentration tertile had increased CAD scores and more often multi-vessel CAD (32.2% of cases) compared to patients in the middle (51.9%, p=0.018) or the lowest tertile (44.8%, p=0.002). Patients in the highest versus lowest IL-6 concentration tertile were more likely to have multi-vessel CAD, after adjustment for risk factors, age, gender and CLMT (adjusted OR: 2.464, 95% CI: 1.165–5.238, p=0.019) or CTT (adjusted OR: 2.523, p=0.015). At ROC C-statistic was a significant half maximum of multi-vessel CAD (AUC=0.698, 95% CI: 0.577–0.809, p=0.003). Circulating IL-6 levels >4.3 pg/ml predicted multi-vessel disease with 64% sensitivity and 67% specificity. Conclusions. Among non-elderly patients with initially diagnosed or suspected CAD, circulating IL-6 levels were associated with CAD severity, suggesting an important link between IL-6 and coronary atherosclerosis.

0624 FIBRINOGEN MODIFICATION BY SHEAR STRESS ACTIVATED PLATELETS

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Background. Laminar shear stress in bulk circulation activates platelets to different levels and may initiate arterial thrombosis at sites of pathological blood flow. The exposition of platelet surface receptors is changed, and storage proteins and low molecular species either stored in dense granules or stored in α-granules are released. The low molecular substance malondialdehyde (MDA) is formed as a consequence of lipid peroxidation and/or of prostaglandin’s metabolism in platelets. MDA is reactive toward amino groups of proteins and nucleic acids, it has been inferred to have mutagenic and cytotoxic roles, possibly to be a participant in the onset of atherosclerosis. In a previous work we studied the influence of oxidative modification of fibrinogen on platelet dynamic adhesion and found significantly lower platelet adhesion on fibrinogen modified with MDA. Aims. The aim of the present study was to determine whether MDA is formed in shear stressed platelets suspended in fibrinogen and if it modifies the fibrinogen molecules. Methods. Blood was drawn from healthy volunteers, who had not ingested any drug for at least two weeks, in accordance with the Ethical Committee regulations of our Institute. Washed blood platelets were isolated by differential centrifugation of blood and resuspended in Tyrode buffer at pH 7.4 in the presence of human fibrinogen (final concentration 1 mg/ml). High shear was applied with a cone and plate analyzer, the Impact-R (DiaMed) in accordance with manufacturer’s manual. Samples (washed platelets - fibrinogen) were placed onto a polystyrene plate onto which a Teflon cone was perfectly fitted. After incubation (10 s) shear was applied (shear rate 1800 s⁻¹) for 1, 5, 15, 30 minutes. The protein carboxyl groups of fibrinogen were derivatized with thiobarbituric acid. MDA formed MDA derivatized with thiobarbituric acid were estimated using dinitrophenylhydrazine, the released serotonin and MDA formation reflected the platelet activation due to shear stress. The most pronounced bands of DNPH-proteins on SDS-PAGE immunooblots were the fibrinogen alpha, beta and gamma chains. Summary/conclusions. Fibrinogen is the most protein vulnerable to oxidative stress products and indeed the fibrinogen molecules are preferable modified with MDA. We found that MDA formed in platelets activated by shear stress and modified platelet proteins and fibrinogen in buffer. According to our previous results malondialdehyde modification of fibrinogen decreases platelet adhesion, therefore the MDA production by platelets activated by shear stress may possibly protect platelets from further activation.

0625 SMOKING AND ADAMTS-13 LEVELS IN HEALTHY MALES

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Background. The procoagulant protein von Willebrand factor (vWF), which has prothrombotic activity, was reported to be elevated in smokers leading to increase incidence of thrombosis. vWF is down-regulated by ADAMTS-13 protease (members of a disintegrin and metalloprotease with thrombospondin 1 repeats). No reports on the effects of smoking on ADAMTS-13 are currently available. Aim. To determine the effects of smoking on ADAMTS-13 antigen and activity levels in healthy Arab smokers in Kuwait. This will clarify the role of ADAMTS-13 in hemostasis and thrombosis, and adds on to the mechanism of thrombosis in smokers. Methods. 200 Arab males were recruited. After obtaining consent, venous blood samples from 80 smoker and 80 non-smoker healthy subjects were collected after asking subjects to fast and refrain from smoking for 8 hours (smokers here were termed “smokers at rest”). Similar sampling was done for 40 smokers, who were asked to smoke one cigarette immediately before taking blood (termed “acute smokers”). For all blood samples, plasma was separated and used to measure ADAMTS-13 antigen and activity levels, as well as vWF collagen binding activity levels using commercial ELISA kits. Results. No difference in ADAMTS-13 antigen level was found between smokers at rest and non-smokers, but ADAMTS-13 and vWF activities were significantly lower in smokers (p<0.018). Compared to smokers at rest, acute smokers showed significantly higher levels of vWF activity and ADAMTS-13 antigen and activity levels (p<0.01). Conclusions. The increase in vWF activity in smokers is an acute mechanism that occurs in response to endothelial injury caused by cigarette consumption. High vWF activity is accompanied by an increase in ADAMTS-13 activity as a natural physiological mechanism to degrade the elevated vWF molecules. If not followed by a decrease in the activities substances in the acute smokers, the reported increase in vWF and constant degradation by ADAMTS-13 result in lower overall levels of both proteins in smokers (at rest) compared to non smokers who do not experience a similar (repeated) injury to the endothelium.

0626 ASSESSING THE IMPACT OF THE GSBA POLYMORPHISM ON FIBRINOGEN A-CHAIN GENE IN PATIENTS WITH STABLE ANGINA: EFFECTS ON SPECIFIC MARKERS OF COAGULATION

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Purpose. Evidence suggests that the GSBA polymorphism on fibrinogen α-chain gene is associated with increased fibrinogen levels in healthy individuals. However, it is still unclear, whether this polymorphism is associated with coagulation/thrombosis in patients with coronary artery disease (CAD). In the present study we examine the impact of this polymorphism on fibrinogen levels, D-dimers levels and plasminogen levels. Methods. The study population consisted of 395 subjects, 246 of which angiographically documented for CAD. The GSBA polymorphism was detected by polymerase chain reaction (PCR) and appropriate restriction enzymes. Fibrinogen levels were measured by immunonephelometry, while plasminogen and D-dimers levels were measured by standard coagulometry techniques. Results. The genotype distribution was GG: 37.8%, GA: 39.4% and AA: 22.8% for patients with CAD, while GG: 33.5%, GA: 44.3% and AA: 22.2% for controls. Patients with CAD had significantly higher fibrinogen levels (mg/dl) than controls (454.7±152.7 vs 384.7±103.7, p=0.0002). However, in patients with CAD fibrinogen levels were not significant higher for GSAA homozygotes vs GS carriers (453.6±131.4 vs 441.1±140.6, p=NS), while similar difference occurred in controls (AA: 385.2±129.4 vs GG+GA: 392.6±103.0, p=NS). Moreover, D-dimers levels (mg/l) were significantly higher in CAD patients with GSAA genotype than controls (409.7±188.2 vs 332.8±199.4, p<0.0001). In addition, there was a significant difference for 58G carriers vs GSAA homozygotes for CAD patients (506.4±418.8 vs 662.2±627.1, p<0.05), but not for controls (AA: 415.6±289.6 vs GG+GA: 355.9±276.5, p=NS). Finally, CAD patients and controls had no significant difference in plasminogen levels (u/ml) (147.6±27.9 vs 118.3±22.9, p=NS).

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112.2±17.2, p=NS), while no significant difference was observed for controls (AA: 112.8±16.7 vs GG+GA: 114.3±25.5, p=NS). Conclusions: Our findings indicate that the G58A polymorphism on fibrinogen a-chain gene affects D-dimers levels in patients with coronary artery disease. These findings provide a possible mechanism by which this polymorphism may affect thrombotic process/coagulation independently of fibrinogen levels and may have important clinical implications.

**6027**

**ALTERED COAGULATION PROCESS IN PATIENTS WITH HYPERTENSION: THE ROLE OF THE G455A POLYMORPHISM**

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**Purpose.** Genetic polymorphism G455A on fibrinogen b-chain gene has been associated with the risk of coronary artery disease. However, it is unknown whether it affects the prothrombotic profile of patients with hypertension (HT). In the present study we examined the impact of this polymorphism on fibrinogen, D-dimers, factor V (FV) and factor X (FX) levels in the aforementioned population. Methods. The study population consisted of 266 HT and 165 non HT. The G455A polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, while circulating fibrinogen were measured by the von Clauss method. D-dimers, FV and FX levels were measured by standard coagulometry techniques. Results. The genotype distribution in non HT and HT was GG: 50.9%, GA: 41.8%, AA: 9.7% and GG: 51.5%, GA: 37.6%, AA: 10.9% respectively. There was no significant difference in fibrinogen levels (µg/dl) between 455AA homozygotes and 455G allele carriers (472±84 vs 448±34, p=NS). Importantly, 455AA genotype presented with much more elevated levels of fibrinogen compared to the GG+GA in HT patients (554±25 vs 414±22, p<0.001). Moreover, HT 455AA homozygotes had significantly increased D-dimer levels (µg/l) compared to 455G allele carriers (640.3±83.6 vs 485.5±27.2, p<0.001). No difference was observed for non HT regarding D-dimers between the 455AA genotype and GG+GA (477±67.4 vs 450±40.7 µg/ml, p=NS). Importantly, 455AA genotype presented with higher FV (%) and FX (%) levels compared to GG+GA in HT patients (133.6±5.8 vs 126.8±26.6, p=NS for FV and 114.6±24.7 vs 114.6±26.6, p=NS for FX). Conclusions. The genetic polymorphism G455A on fibrinogen b-chain gene has a remarkable impact on prothrombotic profile of patients with hypertension, given its effect on fibrinogen, D-dimers, factor V and factor X levels. These findings provide evidence that this polymorphism affects significantly the mechanisms of the coagulation process in hypertensives.

**6028**

**ALTERED ENDOTHELIUM-DEPENDENT RELAXATION INDUCED BY ERYTHROCYTE MEMBRANE FROM SUBJECTS WITH DIFFERENT HAEMOGLOBIN GENOTYPES IN ISOLATED RABBIT CAROTID ARTERIES**

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**Background.** The literature contains conflicting reports concerning changes in vascular reactivity following interaction between erythrocytes and vascular endothelial cells. The goal of this study was to characterize the effect of constituents of erythrocytes from subjects with different Hb genotypes on acetylcholine-induced endothelium-dependent vasorelaxation. Methods. Isometric contractions of ring preparations (5mm long) of rabbit carotid artery (n=6) suspended in 20ml organ baths and bubbled with 95% O2, 5% CO2 were studied pharmacologically using standard in vitro techniques under an initial load of 1g, at 37°C, and pH 7.4. Concentration-dependent contractile responses induced by phenylephrine (PE) as well as relaxation responses induced by acetylcholine (Ach) were examined and their respective EC70 and IC70 values obtained. Acetylcholine (IC70) induced relaxations of pre-constrictions induced by EC70 PE were examined in control rings as well as in rings exposed for 30 minutes to (a) intact washed erythrocytes (b) erythrocyte ghosts and (c) haemoglobin - all obtained from subjects of different haemoglobin genotypes (AA, AS and SS). Arterial rings were exposed to 50µl of each of the erythrocyte constituents at an adjusted haematocrit of 0.6. Results. Ach-induced relaxation was significantly (p<0.05) enhanced by AA erythrocytes (46.2±3.25 and 49.5±2.4% for control and test, respectively). In contrast, AS and SS erythrocytes as well as exposure to Hb did not significantly alter Ach relaxation whereas AS and SS ghosts significantly (p<0.01) attenuated Ach relaxation. Compared with AA, erythrocytes but not Hb from AS and SS subjects elicited significantly greater inhibition of Ach relaxation; furthermore, inhibition of Ach relaxation by erythrocyte ghosts was significantly greater with AS than SS. Conclusions. Our findings show that a membrane-bound factor may account for the genotype-dependent attenuated Ach-induced relaxation following interaction between erythrocytes and vascular endothelial cells.

**6029**

**A SINGLE NUCLEOTIDE POLYMORPHISM ON FIBRINOGEN BETA CHAIN GENES MODIFIES ATEROGENESIS INDEPENDENTLY OF FIBRINOGEN LEVELS**

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**Purpose.** Evidence suggests that impaired coagulation process contributes to atherogenesis and its clinical manifestations. In addition, the role of the genetic variability on fibrinogen beta chain gene on fibrinogen levels is widely speculated. In the present study we sought to examine whether a single nucleotide polymorphism could affect the coagulation cascade beyond its effect on fibrinogen. Methods. The study population consisted of 318 patients with documented coronary artery disease (CAD) and 221 controls. The G455A polymorphism was examined using the polymerase chain reaction (PCR) and the specific HaeIII restriction enzyme. Serum levels of D-dimers, factor (F) V, factor (F) X and FV were measured by standard coagulometry techniques. Results. The genotype distribution was GG: 49.3%, GA: 41.2%, AA: 9.5% for CAD and GG: 55.2%, GA: 85.7%, AA: 9.1% for controls. Through the study (overall) population the G455A polymorphism affected significantly D-dimers levels (µg/l) between the G allele carriers and the AA homozygotes (median: 2.55 [2.57-2.71] vs 2.68 [2.58-2.83], p<0.05), as well as FV (%) (114.6±24.7 vs 126.8±26.6). No effect was observed on FX levels (p=NS). Moreover, there was no significant difference in the studied parameters across the genotypes in the control group (p=NS for all). Conclusively, the trend of the control group, the G455A polymorphism defined D-dimers and FX and FV levels in CAD patients. More specifically, the AA homozygosity was associated with significant increased levels of D-dimers (median: 2.75 [2.50-2.90] vs 2.60 [2.40-2.70]), FV (153.0±20.5 vs 119.5±24.8) and FX (102.4±19.6 vs 90.1±22.8) compared to the G allele carriers (p<0.05 for all). Conclusions. A single nucleotide polymorphism on beta chain fibrinogen gene, regulates D-dimers, factor V and factor X levels in patients with advanced atherosclerosis. Our findings indicate that the G455A polymorphism may affect the coagulation, a responsible underlying mechanism for atherogenesis and this is beyond its effect on fibrinogen.

**6030**

**ADMISSION MEAN PLATELET VOLUME PREDICTS LEFT VENTRICULAR SYSTOLIC DYSFUNCTION IN PATIENTS WITH ACUTE ST-ELEVATION MYOCARDIAL INFARCTION TREATED WITH PRIMARY ANGIOPLASTY**

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**Background.** Left ventricular systolic dysfunction (LVSD) after acute myocardial infarction (AMI) worsens short- and long-term prognosis. Platelet volume mean (MPV), an accurate indicator of platelet reactivity, is an independent predictor of impaired angiographic reperfusion and of both short- and long-term mortality after AMI. Aim. The aim of the study was to investigate the role of admission MPV on LVSD post AMI Methods. The study included 360 patients (pts) with first acute ST-elevation myocardial infarction (STEMI), admitted to the University hospital in Zagreb from January 2001 till December 2007. In all the pts angioplasty of the culprit lesion was performed (only pts with <12 h after the onset of symptoms were included and pts with malignant or inflammatory disease and cardiogenic shock were excluded from the study). Whole group characteristics: 70% man, mean age 63 years, diabetes in 27%, hypertension in 64%, current smoking in 35%, hyperlipidemia in 34%, infant related artery: left anterior descending artery in 43%, left circumflex artery in 14%, right coronary artery in 43%, Killip class ≥1 in 23%, multivessel disease in 54%, TIMI flow: >1 pre PCI in 22%, >1 post PCI in 96%, time to treatment 4.8±3.6 h, MPV, platelet count (PLT) and C-reactive protein (CRP) were obtained at the time of admission.
and samples were processed within 1 h of venipuncture. LVSD (defined as left ventricular ejection fraction ≤50%) was assessed using transthoracic echocardiography (Simpson’s method) within 6 days post AMI. Results. 126 pts (85%) had LVSD. Pts with LVSD were older, more frequently had anterior myocardial infarction, multivessel disease, heart failure on admission and longer time to reperfusion. Peak creatinine kinase and admission troponin were significantly higher in pts with LVSD while CRP, admission creatinine, baseline and final TIMI flow, gender and cardiovascular risk factors were comparable in both groups. MPV was higher in pts with LVSD (8.82 vs. 8.46 fl, p<0.001), and increased with the degree of admission heart failure (5.6, 7.7, 9.32 fl for Killip class I-II respectively, p<0.001) while FLT did not differ between groups. MPV was negatively correlated with FLT (r=-0.3, p<0.001) and positively with peak creatine kinase (r=0.16, p=0.03). ROC curve analysis showed that values of MPV>8.6 fl had 71.4% sensitivity and 57.3% specificity for predicting LVSD. After multivariable adjustment the independent predictors of LVSD were: MPV (OR 1.553, 95% CI 1.079-2.179), age (OR 1.095, 95% CI 1.029-1.165), anterior myocardial infarction (OR 6.194, 95% CI 1.808-20.388), multivessel disease (OR 3.538, 95% CI 1.105-11.333) and peak creatine kinase (OR 1.0006, 95% CI 1.0003-1.0009). Conclusion. Admission MPV is independent predictor of LVSD in pts with STEMI treated with primary angioplasty.

0631 ANALYSIS OF BLOOD COAGULATION PROCESS WITHIN MURAL THROMBI GENERATED UNDER WHOLE BLOOD FLOW CONDITIONS

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Background. Mural thrombus formation at sites of damaged vessel walls is fundamentals for both physiologic haemostasis and pathological intravascular thrombosis. Blood coagulation process playing a role in stabilizing thrombus has been suggested to far be elucidated in classic experimental approaches, such as APTT assays, that evaluate fibrin clot formation in a plasma solution devoid of blood cell components including platelets. However, such classic coagulation assays cannot necessarily reflect the in vivo mural thrombus formation, in which platelet adhesion/aggregation and blood coagulation can upregulate each other under whole blood flow conditions. Aims. We therefore established an invitro assay system that can precisely evaluate the blood coagulation process during mural thrombus formation under whole blood flow conditions. Methods. Using an invitro perfusion chamber system, blood coagulation during mural thrombogenesis under whole blood flow was visually evaluated by confocal laser scanning microscopy (CLMS) as the extent of intra-thrombus fibrin network formation, which was calculated as the ratio of fibrin relative to fibrinogen within mural thrombi generated on a collagen- or von Willebrand factor-coated surface in an immune-fluorescent method. The blood coagulation process within thrombus under flow was also evaluated in detail by the time-course changes of P-selectin expression, tissue factor (TF) accumulation, or thrombin binding on platelets. Results. Analysis by CLMS during perfusion of whole blood anticoagulated to various extent revealed that the size and shape of thrombi was dependent on the amount of intra-thrombus fibrin deposition under high shear rate conditions. The generation of platelet procoagulant activities was confirmed to occur with the sequence of (1) P-selectin expression, (2) TF accumulation, (3) thrombin binding, and eventually (4) fibrin deposition within platelet thrombi. Summary/Conclusions. Our system enables real-time observation of fibrin network formation in mural platelet thrombi, as well as analysis of the functional link between blood coagulation and mural thrombogenesis under whole blood flow conditions. With this approach, we visually evaluated successfully mural thrombogenicity of hemophilia patients with or without addition of activated coagulation factor VII, as well as the antithrombotic effects of 5-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors ("statins") under whole blood flow, an experimental situation most relevant for the in vivo haemostasis and thrombosis.

0632 HEMOSTATIC MARKERS EVALUATION IN A TRIAL OF THROMBOPROPHYLAXIS FOR NEWLY DIAGNOSED MYELOMA PATIENTS TREATED WITH LENALIDOMIDE AND DEXAMETHASONE

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Background. Multiple myeloma (MM) patients receiving lenalidomide and dexamethasone combined therapy have an increased risk of thrombosis. Low-molecular weight heparin (LMWH) and low-dose aspirin (ASA) are used as thromboprophylaxis in these patients. In a prospective, multicenter phase III trial (RV-MM-PI-209) of newly diagnosed MM patients treated with lenalidomide and low-dose dexamethasone during induction therapy, the safety and the efficacy of low-molecular weight heparin (LMWH) or low-dose aspirin (ASA) as anti-coagulant prophylaxis was assessed. Aims. In a group of MM patients enrolled in the RV-MM-PI-209 trial, selected markers of hemostatic system activation were measured in order to evaluate: 1. the biomarkers’ predictive value for thrombosis, and 2. the modulation of the biomarkers by thromboprophylaxis during lenalidomide administration. Methods. Induction treatment consisted of four 28-day cycles of lenalidomide (25 mg d 1-21) and low-dose dexamethasone (40 mg d 1,8,15,22). All eligible patients were randomly assigned to receive LMWH (Enoxaparin 40 mg/d, N=166) or ASA (100 mg/d, N=176) for the duration of the induction therapy. Plasma samples from 36 patients were available for analyses at baseline (T0) before starting treatments (22 LMWH/14 ASA). In addition, plasma samples from 15 study subjects (10 LMWH/5 ASA) were obtained at the end of induction therapy (T1). On all plasma samples we measured prothrombin fragment F1+2 and Tissue Factor (TF), as markers of coagulation activation, and thrombomodulin (TM) as marker of endothelial cell activation. Forty healthy subjects acted as the control group. Results. At baseline, the levels of F1+2 (158±10.7 vs 100±15 nmol/L, p=ns), TF (115±20 vs 69±10 ng/ml; p=0.08) and TM (34±5.7 vs 11±0.9 ng/ml; p<0.001) were increased in the 36 MM patients compared to healthy controls. To evaluate the effect of lenalidomide/dexamethasone therapy, in association with LMWH or ASA, data from patients having samples available at both T0 and T1 were compared. The results showed that a reduction occurred from T0 to T1 for F1+2 (124±13 vs 109±16 nmol/L, p=ns), TF (164±21 vs 64±6.9 ng/ml; p=0.02), and TM (52±4.3 vs 25±2.8 ng/ml; p=ns) plasma levels. In addition, the plasma levels of both F1+2 and TF at the end of the induction therapy were similar to that of control subjects. Differently, at the same time point, the levels of the endothelial activation marker TM were still significantly higher compared to controls. The decrease of F1+2 observed at T1, the marker of thrombin generation, was statistically significant for the subgroup of patients under LMWH (p=0.01), but not in those on ASA. Conclusions. Our data confirm the occurrence of blood coagulation and endothelium activation in MM patients. After induction therapy, the levels of the two blood coagulation makers tended to normalize towards controls’ values. Differently, TM was still significantly higher, suggesting that the influence of the antitumor therapy on endothelium. The analysis of data according to the type of thromboprophylaxis, showed that F1+2 significantly decreased in those patients receiving LMWH, but not in those receiving ASA. No thrombotic events occurred in the analyzed subgroup of patients to allow evaluation the predictive value for thrombosis of these markers.
Potential role of glutamine metabolism in immunomodulation of T lymphocytes by mesenchymal stromal cells

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Background. Mesenchymal stromal cells (MSCs) exert an immune regulatory function and suppress T-cell proliferation, but the mechanisms underlying this property are not completely known. Glutamine, the most abundant free amino acid of the human body, is metabolized by the enzyme glutaminase generating glutamate. However, in situations of glutamine deficiency, the enzyme glutamine synthetase produces glutamine from glutamate. Glutamine regulates cell proliferation by activating protein kinase A and mTOR signaling. Interestingly, inhibition of the mTOR pathway leads to an increased generation of regulatory T-cells. Aims. In this work, we evaluated the potential role of MSCs in the modulation of glutamine levels, as an immunomodulatory mechanism acting upon T lymphocytes. Methods. Peripheral blood CD3+ T-cell from 3 individuals were activated by anti-CD3/CD28 beads and cultured or not with MSC. Following a 5 day period, CD4+ T-cells and CD8+ T-cells were purified and profiled by whole genome microarrays and selected genes validated by RT-PCR. T-lymphocytes from 3 independent individuals were similarly activated and cultured. In addition, we cultivated MSCs alone or in presence of activated T-cell supernatant. Following a 5 day period, glutamine and glutamate levels were analyzed in culture media by hplc. In another experiment T cells and MSC were co-cultivated, as described, and T-cell proliferation was analyzed upon addition of glutamine at different concentrations (0.5, 0.7 and 1.0mM). Results. As expected, proliferation of lymphocytes co-cultured with MSCs was significantly inhibited (cfs). Using the Ingenuity Pathway Analysis, our microarray data revealed that mTOR pathway was down regulated in lymphocytes immunomodulated by MSC. Concordantly, these immunomodulated lymphocytes expressed higher levels of genes associated with regulatory T-cells, such as IL10, FOXP3, CTLA-4 and GITR (validated by RT-PCR). Levels of glutamine were lower in the culture media of T-cells cultivated with MSCs (as compared to those cultured alone), while the levels of glutamate were higher. In line, the expression of glutamine synthetase was increased in immunomodulated lymphocytes. Interestingly, while high levels of glutamine were found in the media of MSCs or activated lymphocytes cultured alone, when the culture media from MSCs cultured alone was substituted by that of activated lymphocytes cultured alone (in the 3rd day of culture), glutamine levels at the last 5th culture day were strikingly reduced, indicating that the inflammatory stimuli provided by the media of activated T-cells lead MSCs to consume the glutamine present in the media. This mechanism can be responsible by the low levels of glutamine found in the coculture of MSCs and lymphocytes. Finally, while the addition of glutamine to cocultures partially restored the proliferation of immunomodulated T-lymphocytes in a dose dependent manner, lymphocytes cultured alone showed no change in cell proliferation by addition of glutamine. We are currently addressing the potential role of MSCs glutaminase, in the consumption of glutamine, by using a glutaminase inhibitor. Conclusions. Our results point to a new immunoregulatory mechanism, by which MSCs would restrict lymphocyte proliferation, trough the consumption and deprivation of glutamine from the surroundings. Finally, reduced glutamine levels would implicate reduced mTOR signaling, contributing to an increase in the generation of regulatory T-cell.

Interleukin-15 skew human monocyte differentiation into CD56+ dendritic cells with cytolytic activity against myeloid leukemic cells

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Background. Recent research has underscored the potential clinical utility of dendritic cells (DCs) for antileukemia immunotherapy. However, current DC-based immunotherapy strategies leave considerable room for improvement, particularly when it comes to the generation of DCs with maximal immunostimulatory potency. We and others have recently reported on a novel, improved protocol for DC generation using interleukin (IL)-15 (IL-15 DCs). Aims. A subpopulation of these IL-15 DCs was incidentally found to express the natural killer (NK) cell-related molecule CD56. The aim of the present study was to determine whether IL-15 DCs also possess NK cell-like effector functions that would allow them to exert direct cytolytic activity against myeloid leukemia cells. Methods. Human peripheral blood monocytes were differentiated into immature DCs for 24-36hr in the presence of granulocyte macrophage colon-stimulating factor and IL-15. CD56+ and CD36- IL-15 DC fractions were separated by positive immunomagnetic selection, and further matured for 18hr in the presence of

Delicate balance between the absolute numbers of antigen-presenting cells and antigen-specific T cells determines the likelihood of successful in-vitro priming of immune responses

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Background. In-vitro generation of primary T cell responses from a naive repertoire against selected antigens (Ag) is essential for the development of adoptive immunotherapeutic strategies. Although possible, the limited robustness of the procedure hampers broad scale clinical application so far. Aims. In this study, we investigated in detail the role of the type of the antigen-presenting stimulator cells and antigen dosing in priming, survival, and expansion of antigen-specific precursor T cells (1prec). Methods. We developed an in vitro model that allows kinetic functional monitoring of activation and proliferation of antigen-specific T cells in the presence of various sources, including monocytes, monocyte-derived dendritic cells (DCs), EBV-LCL and primary leukemic APC at CTL/stimulator (CTL/S) ratios ranging from 1/10 to 1/625 in the presence of variable numbers of infected bystander T cells. Results. Using professional APC like monocyte-derived DCs as stimulator cells, optimal T cell activation was observed at CTL/S ratios critically ranging between 1/1 and 1/10. This phenomenon occurred irrespective of the presence of different numbers of innocent bystander T cells, illustrating that optimal T cell activation is determined by the specific Tprec/S ratio rather than the total cellular R/S ratio. Exposure of individual CTL to higher numbers of stimulators resulted in overstimulation and activation-induced cell death (AICD) of the specific T cells. Within the naive repertoire, specific Tprec frequencies for a single antigen are estimated to be as low as 1 in 1,000,000 to 1 in 10,000. Taken the above data into account, application of the widely chosen R/S ratio of 10/1 for professional specific Tprec seemed to be a low frequency that would correspond to an effective Tprec/S ratio of 1/1000, which may often result in the induction of AICD of the specific Tprec. This was confirmed by the generation of primary immune responses against (completely) HLA-mismatched APC. Using stimulator cells with an inferior APC phenotype (e.g. monocytes or primary leukemic cells), similar dose-response relationships were observed, but the range of optimal Tprec/S ratios was broader and was shifted towards a higher APC amounts per Tprec. Increasing the antigen dose on the APC surface shifted the Tprec/S ratio towards lower amounts of stimulators per Tprec. Conclusions. In conclusion, our data showed that a delicate balance between the absolute numbers of antigen-specific Tprec and antigen-presenting stimulator cells determines the likelihood of successful priming and survival of antigen-specific Tprec.
ence of the Toll-like receptor 7/8-agonist resiquimod. DC-mediated cytotoxicity towards the myeloid leukemic cell line K562 was measured by flow cytometry after overnight co-culture at different effector:target (E:T) cell ratios. Cytotoxicity blocking assays were performed using concanamycin A, an inhibitor of perforin/granzyme B-induced apoptosis, and neutralizing antibodies against tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Results. Flow cytometric immunophenotyping confirmed the expression of CD56 on a subpopulation of DCs (54±3.2±1.5%). Contamination of NK cells was excluded on the basis of phenotypic analysis. Interestingly, CD56+ IL-15 DCs displayed significantly stronger tumoricidal activity towards K562 cells at E:T ratio of 50:1. Compared to their CD56- counterparts, CD56+ IL-15 DCs exerted a direct cytotoxic effect into CD56+ NK cells, with a specific killing activity of 22.5±2.8%.

The observation that IL-15 DCs exert a direct cytotoxic effect into CD56+ DCs endowed with cytotoxic activity against the myeloid leukemic cell line K562. The perforin/granzyme B-pathway, and to a lesser extent TRAIL-induced apoptosis, were implicated in IL-15 DC-mediated cytotoxicity. The observation that IL-15 DCs exert a direct antileukemic action provides strong support for their use in cellular immunotherapy of myeloid leukemia.

0636 EX-VIVO ALLOGENIC STIMULATION SIGNIFICANTLY IMPROVES EXPANSION OF CYTOKINE-INDUCED KILLER CELLS WITHOUT INCREASING THEIR ALLOREACTIVITY ACROSS HLA-BARRIERS

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Background. Cytokine-induced killer (CIK) cells are a heterogeneous subset of ex-vivo expanded T lymphocytes with a mixed T-NK phenotype and endowed with a HLA-unrestricted antitumor activity. CIK cells can be used in the autologous setting of cancer patients or as an alternative to standard donor lymphocyte infusion following allogeneic hematopoietic cell transplant (HCT). CIK cells present a reduced risk to induce graft versus host disease (GVHD) in HLA identical siblings but retain a certain degree of alloreactivity when challenged across major HLA-binders. Even if CIK cells can be efficiently ex-vivo expanded, their expansion rate has been shown to be highly variable, ranging from only few to more than 1000 folds. This variability may be a potential limitation for their effective clinical applications in patients with poor expansion rates. Aims. We evaluated whether alloreactivity of CIK cells might be exploited as a new method to favorably increase their ex-vivo expansion preserving the antitumor ability and safety profile. Our hypothesis is that a timed allogeneic stimulation might provide CIK cells with a proliferation boost and potentially decrease the overall alloreactivity versus hypothetical third party recipients. Methods. Starting from healthy donors (n=7), we compared in parallel the standard expansion protocol of CIK cells, based on IFN-γ, IL2, with a new one including the addition of irradiated allogeneic PBMC (ratio 1:1) as stimulators on day +7 and +14. After 4 weeks we compared expansion rates, antitumor activity and residual alloreactivity across major HLA-binders (assessed by MLR versus third party HLA-mismatched PBMC, different from those used as stimulators during the experimental expansion). Results. Allo-stimulated CIKs (AS-CIK) presented significantly higher expansion rates (median 131 fold, range 24-720) compared to standard controls (median 52 fold, range 9-121, p<0.05). The expansion of the CD5+CD56+ fraction, mainly responsible for the antitumor activity, was significantly higher (2248 fold, range 443-4319) within AS-CIKs compared to controls (403 fold, range 67-1722, p<0.03) (Fig. 1). AS-CIKs retained effective tumor killing ability (64%, 57%, 52% and 48% of specific killing, assessed by flow cytometry after overnight co-culture at different effector:target (E:T) cell ratios to K562). Contamination of NK cells was excluded by flow cytometry after overnight co-culture at different E:T cell ratios. Cytotoxicity blocking assays were performed using concanamycin A, an inhibitor of perforin/granzyme B-induced apoptosis, and neutralizing antibodies against tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). Results. Flow cytometric immunophenotyping confirmed the expression of CD56 on a subpopulation of DCs (54±3.2±1.5%). Contamination of NK cells was excluded on the basis of phenotypic analysis. Interestingly, CD56+ IL-15 DCs displayed significantly stronger tumoricidal activity towards K562 cells at E:T ratio of 50:1 (F=0.0002) and 25:1 (F=0.0065). The perforin inhibitor concanamycin A partially abrogated the cytotoxic effect of CD56+ IL-15 DCs (-51.8±11.7%). A further significant decrease in cytotoxicity was obtained by treatment with anti-human TRAIL antibodies (-22.5±2.8%).

Summary/Conclusions. Here, we describe the unique ability of IL-15 to promote rapid differentiation of monocytes into CD56+ DCs endowed with cytotoxic activity against the myeloid leukemic cell line K562. The perforin/granzyme B-pathway, and to a lesser extent TRAIL-induced apoptosis, were implicated in IL-15 DC-mediated cytotoxicity. The observation that IL-15 DCs exert a direct antileukemic action provides strong support for their use in cellular immunotherapy of myeloid leukemia.
lectins receptors significantly suppressed the proportion of pNK cells and the intensity of CD69 upregulation on pNK cells after coincubation with CTV-1 cells after 24 hours and 48 hours. In contrast, blocking of Ig-SF like receptors had no effect even when combined with the blockade of C-type lectins (Fig). CD69 is a known triggering ligand for human NK cells and we hypothesise that its presence and degree of expression is a marker of the pNK readiness to lyse tumor target cells.

**Conclusion.** C-type lectin are the predominant NCRs involved in the process of NK cell priming by tumor cells while the Ig-SF like receptors have little or no role in the priming process.

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**IL-10 and Regulatory T Cells Limit T Cell Responses After Transcutaneous Immunization**

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The imidazoquinoline derivate imiquimod induces inflammatory responses and protection against transplanted tumors when applied to the skin in combination with a cognate peptide epitope (transcutaneous immunization, TCI). Investigating the influence of UV irradiation on the induction of a peptide-specific CTL-response we found that combining TCI with low dose UV-B boosted the CTL-response and induced potent memory formation. For UV-irradiation the induction of regulatory as well as NK-T cells and the release of suppressive cytokines like IL-10 and IL-4 has been described. As we were interested in the mechanisms behind supporting and inhibiting factors we depleted FoxP3+ regulatory T cells and found that specific CTL-responses were greatly enhanced. In in vivo studies it has been published that natural occurring as well as induced Treg produce IL-10 to control immune responses. Here we can show that in our immunization protocol Treg mediated immune suppression is only partly dependent on the release of Treg-derived IL-10 which itself inhibits immune response formation as could be shown in experiments with IL-10/- mice or application of an anti-IL-10-receptor-Ab. As the manipulation of NK-T cells seems to play a substantial role in UV-induced suppression we immunized a NK-T cell free system and induced increased immune responses showing suppressive capacities for this cell type. Understanding the structure behind UV-enhanced TCI could lead to further improvements and advanced vaccination protocols against tumors and persistent virus infection. arandolopez@uni-mainz.de

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**Molecular Mechanisms Involved in T-cell Suppression by Regulatory T Cell Generation by Mesenchymal Stromal Cells**

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**Introduction.** In recent years, Mesenchymal Stem Cells (MSCs) have aroused the attention from scientific community for their capacity to suppress the T-cell proliferation and regulatory T-cells (Tregs) generation. These immunological properties of MSC attracted the interest of basic and clinical investigators, in light of its potential therapeutic use in different immunological diseases. Nevertheless, successful translation into clinical practice requires further dissection of the ways by which MSCs modulate the signaling pathways activated on T-lymphocytes. **Aims.** Explore the molecular mechanisms underlying the induction of T-cell suppression and Tregs generation by MSCs. **Methods.**
Peripheral blood T-lymphocytes from 3 individuals were activated by anti-CD3/CD28 beads, cultured in the absence or the presence of MSC. Following a 5 day period, CD4+ lymphocytes were purified and profiled by whole genome microarrays. Real-time PCR was used for evaluations. MSCs inhibited the proliferation of activated T-lymphocytes, as compared to cells cultured alone. The proliferative suppression was clearly paralleled by a general transcriptional repression of components related to TCR signaling and to cell cycle progression. For instance, transcript levels of major components mediating TCR signaling, such as, CD3, LCK, Vav, ZAP70, LAT and GRB2 and controlling cell cycle progression trough the G1 phase, such as Cyclins D1 and E, and corresponding CDK4 and CDK2 kinases, were all repressed in lymphocytes co-cultured with MSCs. Interestingly, the control of TCR signaling is not only involved in the suppression of cell proliferation but also in the induction of a Treg, as contacted TCR stimulation leads to a loss in Foxp3 inducibility. Moreover, the antagonism of the continued TCR stimulation over Foxp3 expression would be mediated by the PI3K/AKT and mTOR signaling pathways, as inhibition of these pathways in activated T-cells induces transcriptional changes driving the generation of Tregs phenotype. Strikingly, our results show that these pathways are clearly transcriptionally repressed on activated T-cells cocultured with MSCs, with the down-modulation of transcripts that include central components of these pathways, namely, the catalytic subunit of PI3K, PDK1, AKT1 and 2, FKBPA1, among others. Moreover, we have evidences that upon coculture with MSCs, canonical NF-κB pathways signaling on activated T-cell is inhibited and substituted by noncanonical signaling and that this correlates with the acquisition of Treg-related genes. The general transcriptional repression of components of TCR, PI3K/AKT and mTOR signaling pathways in activated T-lymphocytes cocultured with MSCs, indicates that a molecular mechanism with a broader action would be implicated in the suppression of the proliferation and the concomitant induction of Tregs. In line with this, our results provide evidences that MSCs can modulate the expression of crucial components of the epigenetic machinery in activated T-cells leading to the general repression observed by us. Conclusion. Our results shown that several pathways related to T-cell activation, proliferation and involved with the induction of a regulatory phenotype, are modulated in lymphocytes, upon coculture with MSCs. These findings are useful to understand the molecular mechanisms involved in T-cell immunomodulation by MSCs and help further studies to ensure the success on the clinical use of these cells.

**0641**

**A UNIQUE IMMUNOTHERAPEUTIC MODALITY BY USING A LEUKEMIC PLASMACYTOID DENDRITIC CELL LINE (PMDC05) AS POTENT ANTIGEN PRESENTING CELL**

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**Background.** Although cellular immunotherapy based on antigen-specific cytotoxic T lymphocytes (CTLs) against tumors including leukemia and severe infections is a promising strategy, one of the pivotal issues is a hardship in constant supply of high quality antigen presenting cells (APCs) for generating CTLs against tumor or pathogen-associated antigens. We established a leukemic plasmacytoid dendritic cell (pDC) line (PMDC05) with potent antigen presenting capacity from leukemia cells of a HLA-A*0201/*2402 patient with pDC acute leukemia. PMDC05 possessed a considerable antigen presenting ability to naive T cells, which was enhanced by culturing with IL-8 or influenza virus and especially by LPS. **Aims.** In order to establish PMDC05-based tumor immunotherapy, we investigated whether PMDC05 could be efficiently used for generating CTLs specific for antigens of leukemia cells or pathogens. In addition, in order to elevate the efficiency of PMDC05-based anti-tumor immunotherapy, we evaluated the effectiveness of newly synthesized inhibiting agent for indoleamine-2,3-dioxygenase (new IDO inhibitor) for potentiating antigen presenting ability of PMDC05. **Methods.** PMDC05 cells, which was stimulated with 0.1 µg/mL LPS and loaded with 10 µg/mL WT1 peptides (HLA-A*2402-restricted, modified-type 9-mer peptide; CUTFQM4N) or CMV pp65 peptides (HLA-A*2402-restricted, 9-mer peptide; OYDPAVAAL) for 24 hours, was co-cultured with allogeneic CD8+ T cells which were purified from peripheral blood mononuclear cells (PB-MNCs) of HLA-A*2402+ healthy donor (PMDC05:CD8+ Tcells = 1:10), 50 U/mL IL-2 was added to the co-culture at day 2, and IL-2 as well as 10 ng/mL IL-7 were added every 3 days thereafter. Induction of WT1 or CMV-specific CTLs was evaluated by flow cytometry and using HLA-A*2402 WT1 tetramer or CMV tetramer every week. In addition, PMDC05 cells were treated with new IDO inhibitor during the stimulation with LPS and IFN-γ and used as stimulator cells in mixed leukocyte culture (MLC) using PB-MNCs as responder cells. MLC was performed in both CFSE-based proliferation and ‘H-thymidine incorporation methods. Results.** PMDC05 began to induce WT1 tetramer-CD8+ T cells at week 4 and the percentage of WT1 tetramer T cells in CD8+ T cells rose to more than 75% at week 7. Likewise, CMV tetramer-CD8+ T cells were amplified in CD8+ T cells co-cultured with CMV peptide-pulsed PMDC05. By treating PMDC05 cells with new IDO inhibitor, antigen presenting ability was increased in both CFSE-based proliferation and ‘H-thymidine incorporation methods. **Conclusions.** These data suggested that PMDC05 could be efficiently used for generating CTLs specific for tumor or pathogen-associated antigens and newly synthesized IDO inhibitor could be applicable for enhancing the antigen presenting activity of PMDC05. These findings revealed that PMDC05 cells in combination with new IDO inhibitor could be a promising strategy in cellular immunotherapy for tumors and severe infections.

**0642**

**IMMUNOTHERAPY OF RECURRENT B-CELL MALIGNANCIES IN PEDIATRIC PATIENTS WITH THE TRIFUNCTIONAL ANTIBODY FBTA05 (ANTI-CD3 X ANTI-CD20)**

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**Treatment options for B cell malignancies in pediatric patients refractory to standard therapy are limited.** Novel treatment approaches are urgently required. Bispecific antibodies, especially in combination with allogeneic donor lymphocyte infusion (DLI) and donor lymphocyte infusion (DLI) represent a highly attractive therapeutic concept to direct immunity towards tumor cells. Seven children (three with relapsed diffuse large B-cell lymphoma (DLBCL), one with mature Burkitt lymphoma, two with mature B-cell acute leukemia and one with B-cell acute lymphoblastic leukemia) were treated in individual settings with escalating doses of FBTA05. All patients were extensively pretreated and presented refractory to standard treatment (radiation, chemotherapy) including rituxinib. Five children received HLA-identical allogeneic SCT before FBTA05 treatment. In two patients FBTA05 application was followed by DLI. In one child with DLBCL FBTA05 and DLI were combined with lenalidomide in a second treatment cycle. For safety reasons dose escalation always started with 10 µg followed by 20 µg and 50 µg every third day. Thereafter weekly applications (100-300 µg) were performed. Due to tumor progression before start of therapy daily application of FBTA05 were performed in three patients up to maximum doses of 200, 500 and 1000 µg, respectively. Six of the seven children displayed a clinical response: five stable diseases and one complete response (CR). Remarkably, in this patient a CR even in the bone marrow was achieved without SCT and DLI. Overall survival is in the range of 85 up to 551 days (updated at time of presentation). Three out of the seven children died due to relapse or tumor progression. FBTA05 infusions were well tolerated by all children. Adverse events were restricted to fever and chills and could be managed by supportive treatment. Also the combination of lenalidomide and FBTA05 was well tolerated with nausea and increase of pre-existent leucocytopenia during the first cycle (10 and 17 days daily), while a dose of 5 mg daily in the following cycles was well tolerated. In only one patient, human anti-mouse antibodies were detectable. Importantly, this patient could be safely treated with
two additional applications of FBTA05. The cytokine profile was characterized by transient increase of IL-6, IL-8 and IL-10. Plasma concentrations of FBTA05 strictly correlated with the corresponding dosing schedules with up to 0.19 µg/mL after daily escalating applications of FBTA05 up to 1,000 µg accompanied by the rapid clearance within few days. Graft versus host disease (grade III-IV) developed in two patients on one case after DLI, but could be controlled by further immunosuppressive therapies. Based on these encouraging results, FBTA05 shall be further tested in children in a clinical study. Currently, a phase I/II study in combination with DLI in adult patients with low and high grade B-cell lymphoma after allo-SCT (STP-LYM-01-V01) is performed.

**0643**

A NOVEL SINGLE CHAIN P53TCR PREVENTS GVHD IN A HUMANIZED MOUSE MODEL OF ADOPTIVE T CELL TRANSFER

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**Background.** Adoptive cell therapy with T cells retrovirally transduced with tumor-associated antigen (TAA) specific TCRs is a promising approach for immunotherapy in patients with hematological malignancies. The TAA p53 is over-expressed in approximately 50% of human tumors. One barrier to the development of T cell-based immunotherapies is the presence of high-affinity tumor specific TCRs in the patient due to self-tolerance. We have reported that HLA-A*0201 (A2.1 transgenic mice can be used to circumvent self-tolerance to universal human TAA and to generate efficient tumor-reactive CTL. We used A2.1 transgenic mice, in which the mouse CD8 molecule cannot efficiently interact with A2.1 to generate a high-affinity, CD8-independent p53(264-272) specific TCR. Retroviral expression of CD8-independent p53-specific TCR into T cells, allowed CD8+ T lymphocytes to acquire a broad tumor-specific CTL activity but also redirected CD4+ T cells into potent tumor-reactive, p53-specific T helper cells. However, a particular safety concern with TCR gene transfer, is the formation of mixed TCR heterodimers between the introduced TCR α and β chains with the endogenous TCR chains, resulting in the potential generation of autoreactive T cells. **Aims.** Our aim is to optimize p53TCR construct to prevent mixed dimers formation. **Methods.** To reduce the formation of TCR mixed dimers an inter-chain disulfide bond (Cys.) between the TCR α and β chain constant domains was introduced. We further improved the expression level of p53 TCR transgene using codon-optimization (Opt.) of the TCR sequence in retroviral vector containing the self-cleaving 2A virus-derived peptide element. To prevent the formation of mixed TCR dimers, we engineered a novel p53(264-272)-specific Opt.Cys single chain (sc) TCR construct. The safety of engineered Opt.Cys p53TCR constructs was assessed in a humanized mouse model of adoptive T cell transfer. **Results.** Mouse T cells transduced with p53(264-272)A2.1-Opt.Cys TCR showed higher expression levels of the introduced TCR as compared to p53(264-272)-specific WT TCR. Importantly, p53(264-272)A2.1-Opt.Cys and sc TCR transduced CTL recognized and killed a wide variety of malignant A2.1 tumor cells with altered p53 expression, but not p53-deficient A2.1 cells more efficiently as compared to CTL transduced with the WT p53(264-272)-specific TCR. However, when p53(264-272)A2.1-Opt.Cys TCR transduced mouse T cells are adoptively transferred into p53-deficient partially humanized (A2Kb) mice, under conditioning-induced lymphopenia, expansion of infused T cells following high dose IL-2 administration is associated with the development of lethal autoimmunity similarly to mice receiving p53(264-272)-specific WT TCR transduced T cells due to the formation of self-reactive TCRs. In contrast, mice receiving T cells transduced with p53(264-272)A2.1-Opt.Cys scTCR did not develop any sign of GVHD. **Conclusions.** Our data demonstrate that p53(264-272) TCR gene transfer-induced off-target autoimmunity observed in preclinical mouse model could be prevented by codon-optimized p53(264-272) engineered scTCR, suggesting that sc p53TCR may represent a new and safe approach for TCR-based gene therapy of p53-associated hematological malignancies.
**0645**

**A SINGLE INJECTION OF AAV-8 VECTOR EXPRESSING IL-24 EFFICIENTLY SUPPRESSES TUMOR GROWTH MEDIATED BY MULTIPLE ANTI-TUMOR MECHANISMS IN MLL/AF4 POSITIVE ALL MODEL MICE**

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Mixed-lineage leukemia (MLL)/AF4 positive acute lymphoblastic leukemia (ALL) is a very common leukemia in infant and is associated with high relapse rate and poor prognosis even after allogeneic bone marrow transplantation (allo-BMT). The resistance to graft-versus-leukemia (GVL) effect may be responsible for the poor effect of allo-BMT on MLL/AF4 positive ALL. Interleukin 24 (IL24) selectively induces apoptosis in cancer cells without harming normal cells. It also exerts immunomodulatory and anti-angiogenic effects, as well as potent antitumor bystander effects, making it an ideal candidate for use in a new anti-cancer gene therapy. Here, we examined the feasibility of antitumor bystander effects, making it an ideal candidate for use in a new anti-cancer gene therapy. We examined the feasibility of antitumor bystander effects, making it an ideal candidate for use in a new anti-cancer gene therapy. Here, we examined the feasibility of antitumor bystander effects, making it an ideal candidate for use in a new anti-cancer gene therapy.

**0646**

**TGF-β1 MODULATES LPS-INDUCED CYTOKINE/CHEMOKINE PRODUCTION AND INHIBITS NF-κB, ERK AND P38 ACTIVATION IN DENDRITIC CELLS**

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**Background.** There is growing evidence that dendritic cells (DC) can not only immunogenic but also tolerogenic, both intrathymically and in the periphery. Previously, we have found that, compared with immature DC (iDC), TGF-β1-treated DC (TGβ-DC) are resistant to maturation stimulus-lipopolysaccharide (LPS) and may have correlation with the downregulation of Toll-like receptor (TLR)-4 expression. Moreover, we also have demonstrated that recipient-derived TGβ-DC induce major histocompatibility complex (MHC)-specific tolerance in a murine bone marrow transplantation (BMT) model. However, the molecular mechanisms underlying the phenotypical and functional changes in TGβ-DC upon LPS stimulation have not been clearly elucidated. **Aims.** To analyze whether TGF-β1 affected the production of cytokines/chemokines and proteins in the TLR4 signal transduction pathway following LPS stimulation. **Methods.** C57BL/6J murine bone marrow cells were cultured with different cytokines combination to generate iDC (GM-CSF only) and TGβ-DC (GM-CSF+TGF-β1). Afterwards, they were stimulated by lipopolysaccharide (LPS) for 2 days to induce nuclear translocation of nuclear factor (NF)κB and matura-

**Results.** The concentrations of murine IL-12p70, IFN-γ, IL-18 and IL-10 in culture supernatants were assayed by ELISA. The mRNA levels of CCL2, CCL3, CCL5, CXCL10 were detected by reverse-transcription polymerase chain reaction (RT-PCR). We used electrophoretic mobility shift assay (EMSA) to determine the NF-κB activity in DC. ERK1/2 and p38 mitogen activated protein kinases (MAPKs) protein expression were checked by Western blot analysis. **Results.** In the resting state, both two types of DC produced low levels of cytokines. **Conclusions.** TPB stimulation induced cytokine production, but with a distinctly different pattern for each subset. The production of IL-12p70 in TGβ-DC was significantly less than that of iDC (115.4±15.2 pg/ml vs. 157.0±29.7 pg/ml, P<0.01), but the production of Th2 cytokine-IL-10 was significantly higher (132.1±17.5 pg/ml vs 75.1±16.6 pg/ml, P<0.05). We also found that levels of anti-inflammation chemokines mRNA, CCL2, CCL3 and CXCL10 increased earlier and remained relatively higher in iDC than in TGβ-DC after LPS stimulation. In contrast, CCL5 mRNA was expressed at comparable levels between the two subsets of DC. The results suggested that NF-κB DNA binding activity was significantly increased in iDC in response to LPS, but the addition of TGF-β1 to DC decreased NF-κB binding. Furthermore, TGF-β1 was effective in suppressing LPS-induced activation of ERK1/2 and p38 kinase, the level of phosphorylation of ERK1/2 and p38 kinase were lower than iDC. **Conclusions.** Our findings reveal that TGF-β1 modulates the secretion of inflammatory cytokines/chemokines in DC. These effects may result from interfering with the activities of key elements of TLR4 pathway, such as NF-κB, ERK1/2 and p38 MAPKs. This study strengthens the notion that TGβ-DC are a unique type of tolerogenic DC exhibiting some distinct characteristics.
CLONAL EVOLUTION WITH CHRONIC LYMPHOCYTIC LEUKEMIA DEMONSTRATES

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Background. The difference in clinicobiological behavior of chronic lymphocytic leukemia (CLL) can be related to genetic aberrations present either at diagnosis or acquired during disease course. This clonal evolution has been studied by means of conventional cytogenetic analysis (CCA) and fluorescence in situ hybridization (FISH). However, SNP-arrays enable to investigate clonal evolution at a higher resolution. Aims. This study aimed to characterize clonal evolution using different approaches and to identify a possible association with disease progression, i.e. therapy initiation. Methods. Patient selection met following inclusion criteria: (i) diagnosis of CLL, (ii) at least two available stored samples, (iii) second sample (S2) obtained at least one year after the first (S1), and (iii) patient untreated at both times of sampling. Sequential samples were investigated by CCA, FISH and Affymetrix cytogenetics whole-genome 2.7M arrays on peripheral blood or bone marrow samples. Clinical and biological data were available for all patients. Results. Fifty-three patients fulfilled the selection criteria. The median interval between sampling was 41 months (range 13-102 months). Male/female ratio and median age at diagnosis were 32/21 and 58.4 years (range 30.1-82.8 years), respectively. Patients presented with more advanced Binet stages at S2 vs. S1 (A/B/C in 34/12/7 vs. 47/5/1 patients, respectively). In addition, lymphopenia was higher and lymphadenopathy, splenomegaly, thrombocytopenia, anemia and hypogammaglobulinemia were more frequent at S2. IGHV was unmutated in 15 and mutated in 32 patients. Treatment was initiated in 33 patients after a median duration of 51 months (range 19-173 months) after diagnosis and 1 month (range 0.5-4 months) after S2. Karyotyping revealed clonal evolution in 17/53 cases: acquired aberrations included unbalanced translocations (n=7), del(13)(q14) (n=4), del(11)(q22;q23) (n=4), del(17)(p13) (n=2), del(6)(q) (n=2) and balanced translocations (n=2) and various other aberrations (n=5). In contrast, FISH using the CLL-4 probe panel revealed clonal evolution in only 11 cases. All cases with evolution by FISH, showed del(13)(q14) either as a new or additional subclone. The del(13)(q14) was accompanied by a new del(11)(q22q23) and del(17)(p13), in one case each. Whereas array analysis detected more gains at S1 vs. S2 (9009 vs. 7220 segments, respectively including copy number variations), losses were predominantly found at S2 (n=1295 vs. 2907 segments at S1 and S2, respectively). Recurrent losses were located in the regions 14q14.3, 11q22-23 and 17p13 (n=11/18, 5/7, 0/2 patients at S1/ S2, respectively). Losses of 8p23-11.23, 15q11.2, 22q11.23, 9p22.1, 9p23-21.3 and 14q21.3-22.1 (n=9/5, 5/3, 1/3, 2/2, 2/1 patients at S1/S2, respectively) were also recurrent. In addition, the size of the losses was larger at S2 vs. S1 and in the cohort of patients with impending need vs. without need for therapy; i.e. the mean number of segments lost in patients without vs. with need for therapy at S1/S2 was 18/31 vs. 54/66 in general, 3/12 vs. 11/19 for 14q14.3, and 0/8 vs. 0/13 for 11q22-23, respectively. Conclusion. The present study confirms the occurrence of clonal evolution, especially losses in CLL. Further analysis is ongoing, in order to elucidate the clinical significance of clonal evolution in CLL.

Figure 1. SLL lymph node double stained for CD38 and Ki67.
lymphocytic leukemia (CLL) patients with unchanged peripheral blood mononuclear cells isolated from 9 untreated CLL samples and 2 healthy donors were enriched in CD19+ B cells and subsequently stimulated with a F(ab’2) anti-human IgM or with a F(ab’2) anti-human IgD, at a final concentration of 10 µg/ml. Following 24 and 48 hours of incubation, total RNA was extracted from unstimulated (US) and stimulated (S) samples for miR profiling analysis, performed using the GeneChip miRNA Affymetrix arrays. An unsupervised clustering was applied to evaluate samples responsiveness to BCR ligation. To identify differentially expressed miRs between US and S cases, a t-test retaining only probesets with a p-value <0.05 and a fold change >1.5 was used. Results. An unsupervised approach highlighted that, after both IgM and IgD stimulation, healthy donors clustered apart from CLL cases, suggesting a differential miRs expression pattern in healthy and leukemic B lymphocytes in response to BCR engagement. Based on these findings, we performed a t-test to compare US and S cases. In agreement with the unsupervised analysis, this approach showed a homogeneous signature associated to stimulation in B cells isolated from healthy donors and allowed to identify specific sets of miRs differentially expressed following IgM and IgD ligation, respectively. In CLL, we observed the modulation of several miRs both at 24 and 48 hours of IgM cross-linking, while miR expression changes occurred later, specifically at 48 hours after IgD stimulation, suggesting a delayed activation in this context. Remarkably, miRs selected in CLL S cases were different from those identified in healthy donors, confirming a distinct miR regulation in BCR signaling of these samples. Conclusions. Our study reveals a differential miR expression pattern following IgM and IgD ligation and suggests that distinct mechanisms regulate BCR signal transduction at the physiological and pathological level. Further investigations to combine miR and gene expression profiles obtained from the same samples are currently underway with the aim of identifying putative miR targets.

0650

BCR STIMULATION INDUCES A DIFFERENTIAL MICRORNA (miR) PROFILING BETWEEN B LYMPHOCYTES DERIVED FROM CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS AND HEALTHY DONORS

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Background. MicroRNAs (miRs) are small non-coding RNAs that modulate the expression of genes at the post-transcriptional level, playing a pivotal role in many physiological and pathological processes. In lymphocyte ontogenesis, an involvement of miR-181a and miR-150 in B- and T-cell development has been documented. Moreover, in chronic lymphocytic leukemia (CLL) several miRs are associated with disease pathogenesis and/or outcome. Aims. In order to investigate a potential role of miRs in BCR stimulation, we evaluated the expression of these small RNAs following IgM and IgD cross-linking in B cells, as well as in healthy B lymphocytes. Methods. Peripheral blood mononuclear cells isolated from 9 untreated CLL samples and 2 healthy donors were enriched in CD19+ B cells and subsequently stimulated with a F(ab’)2 anti-human IgM or with a F(ab’)2 anti-human IgD, at a final concentration of 10 µg/ml. Following 24 and 48 hours of incubation, total RNA was extracted from unstimulated (US) and stimulated (S) samples for miR profiling analysis, performed using the GeneChip miRNA Affymetrix arrays. An unsupervised clustering was applied to evaluate samples responsiveness to BCR ligation. To identify differentially expressed miRs between US and S cases, a t-test retaining only probesets with a p-value <0.05 and a fold change >1.5 was used. Results. An unsupervised approach highlighted that, after both IgM and IgD stimulation, healthy donors clustered apart from CLL cases, suggesting a differential miRs expression pattern in healthy and leukemic B lymphocytes in response to BCR engagement. Based on these findings, we performed a t-test to compare US and S cases. In agreement with the unsupervised analysis, this approach showed a homogeneous signature associated to stimulation in B cells isolated from healthy donors and allowed to identify specific sets of miRs differentially expressed following IgM and IgD ligation, respectively. In CLL, we observed the modulation of several miRs both at 24 and 48 hours of IgM cross-linking, while miR expression changes occurred later, specifically at 48 hours after IgD stimulation, suggesting a delayed activation in this context. Remarkably, miRs selected in CLL S cases were different from those identified in healthy donors, confirming a distinct miR regulation in BCR signaling of these samples. Conclusions. Our study reveals a differential miR expression pattern following IgM and IgD ligation and suggests that distinct mechanisms regulate BCR signal transduction at the physiological and pathological level. Further investigations to combine miR and gene expression profiles obtained from the same samples are currently underway with the aim of identifying putative miR targets.

0651

CLONAL EVOLUTION IN CHRONIC LYMPHOCYTIC LEUKEMIA: ANALYSIS OF CLINICOBIOLGIC CORRELATIONS IN 105 PATIENTS

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Background. Clonal evolution (CE) involving chromosomes 17p, 11q, 6q, and 12 was reported in 15-42% of Chronic Lymphocytic Leukemia (CLL) cases using conventional karyotyping or fluorescence in situ hybridization (FISH). The incidence of this phenomenon depends on the length of follow-up and on the number of probes used for interphase FISH analysis. Attention was recently devoted to 14q32 translocation involving the immunoglobulin heavy chain gene (IGH). This aberration was found in 6-19% of CLL patients at diagnosis and was associated with therapy-demanding disease and inferior outcome. The incidence of this aberration at CE is presently unknown. Aims. To analyze the incidence, characteristics and clinicobiological significance of CE including 14q32 translocations in CLL. Methods. 105 patients seen at our institution between 1995 and 2004 were analyzed sequentially by FISH including 14q32 translocations in CLL. Results. 105 patients seen at our institution between 1995 and 2004 were analyzed sequentially by FISH with the following probes: 13q14/D13S25, 11q22/ATM, 17p13/TP53, #12-centromere and 14q32/IGH break-a-part probe. FISH analysis was performed on diagnosis or before 1st line treatment. FISH was repeated at 4-6 year intervals in patients receiving ≤1 line of treatment. In relapsed patients who started 2nd line treatment, FISH was performed sequentially before administration of the 2nd line and before each subsequent line of therapy. These 105 patients fulfilled the following criteria: diagnosis of CLL based on morphology and immunophenotyping; successful FISH analysis at diagnosis and during follow-up (cases with t(11;14)(q13;q32)/BCR-IGH were excluded); clinical record available for revision. Results. The median follow-up of the entire series was 78 months (range 12-180 months). CE was observed in 15/105 patients after 24-170 months (median 64). Recurring aberrations at clonal evolution were 14q32/IGH translocation in 7 patients;
**Background.** Proliferation of chronic lymphocytic leukemia (CLL) cells may be influenced by antigenic stimulation and accessory signals from the microenvironment. Indeed, these factors induce different effects in distinct subgroups of patients, thus sustaining clinical heterogeneity of the disease. Aims. To investigate CLL responsiveness to B-cell receptor (BCR) stimulation, we evaluated the gene expression profile upon IgD cross-linking in different classes of patients, subdivided on the basis of their clinical features; (i) mutation status of the IgH locus; (ii) clinical outcome (CLL survival); survival after the development of CE was 32 months (standard error 8.5). Conclusions. (i) Unmutated poor prognosis CLL and shorter time to chemorefractoriness (TTCR) was noted in 15 patients with CE (p=0.033 and 0.0046, respectively). Survival after the development of CE was 32 months (standard error 8.5). (ii) The 14q32/IGH translocation may be detected earlier in BM or LN samples; (iii) CE in unmutated poor prognosis CLL patients with CE (p<0.0001). The 14q32/IGH rearrangement was associated with 14q32/IGH in 3/7 patients, one of whom also developed a biallelic 13q14 deletion. CE was detected in 15/58 pre-treated patients; to the contrary none of 47 untreated patients developed CE (p=0.0001). The 14q32/IGH rearrangement was detected after 1-4 lines of treatment (median 3 lines). In 3/7 cases with 14q32/IGH translocation BCL2 was the identified partner. In two cases the appearance of 14q32/IGH translocation was first detected in the bone marrow (BM) or in the lymph node (LN) and 13-58 months later in the peripheral blood (PB). ZAP70+ and high risk cytogenetics predicted for the occurrence of CE with borderline statistical significance (p=0.05 and 0.07, respectively). A shorter time to first treatment (TTT) and shorter time to chemorefractoriness (TTCCR) was noted in 15 patients with CE (p=0.033 and 0.0046, respectively). Survival after the development of CE was 32 months (standard error 8.5). Conclusions. (i) 14q32/IGH translocation may represent one of the most frequent aberrations acquired during the natural history of CLL; (ii) The 14q32/IGH translocation may be detected earlier in BM or LN samples; (iii) CE including 14q32/IGH translocation occur in pre-treated patients with short TTT and TTCCR; (iv) survival after CE is relatively short.

**0652**

**ULTRA DEEP SEQUENCING OF IMMUNOGLOBULIN REARRANGEMENTS OF SEQUENTIAL SAMPLES FROM PATIENTS WITH B-CLL DEMONSTRATES CLONAL EVOLUTION**

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**Background and Aims.** B-cell chronic lymphocytic leukaemia (CLL) is the most common leukaemia in the Western World. It is characterised by a chronic relapsing course and the development of chemotherapy resistance. There is mounting evidence that the immunoglobulin heavy chain (IgH) locus plays a central role in antigenic drive and that this may be important for disease maintenance and progression. Previous studies using next generation amplion sequencing (NGAS) of the IGH locus have identified small sub-clones in patients with hypermutated IgH. In this study, we focused on unmutated poor prognosis CLL and used NGAS to study clonal evolution of the IgH locus in sequential samples of the same patients. Methods. We amplified the clone-specific VDJ rearrangement in the immunoglobulin heavy chain using published Biomed consensus primers on sequential samples taken at diagnosis, after first treatment and at subsequent relapse on 4 CLL patients with an unmutated IgH. These products were sequenced on a 454-FLX (Roche Diagnostics). Resulting sequences were grouped using a Perl script to identify recurring reads. Reads of greater than 100 copies were analysed using Jalview and IGMT. Reads present less than 100 times were excluded from the analysis. Results. An average of 30000 reads were obtained for each sample. Within each sample we detected a dominant clone representing approximately 60% of all reads included in the analysis. In addition to this clone, multiple productive rearrangements were also identified when aligned to the germline using IGMT. These were present in a minority of reads. All sub-clones were clonally related to each other and the frequency of both dominant and additional sub-clones remained constant over time in the different samples despite treatment. Interestingly, one patient with an unmutated V1-69 dominant clone was found to have a hypermutated V1-69 subclone present at all three time-points. Conclusions. Together the data suggests that, in unmutated CLL, the leukaemic population consists of a mixture of clonally related dominant and minor IgH clones. The composition and proportion of these IgH clones remains remarkably stable over time. Further analyses of sequential samples are on-going.

**0653**

**GENOMIC AND FUNCTIONAL ANALYSES OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS FOLLOWING IgD STIMULATION**

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**Background.** Proliferation of chronic lymphocytic leukemia (CLL) cells may be influenced by antigenic stimulation and accessory signals from the microenvironment. Indeed, these factors induce different effects in distinct subgroups of patients, thus sustaining clinical heterogeneity of the disease. Aims. To investigate CLL responsiveness to B-cell receptor (BCR) stimulation, we evaluated the gene expression profile upon IgD cross-linking in different classes of patients, subdivided on the basis of the IGHV configuration and the clinical outcome (CLL survival). Results were validated at the functional level using several in vitro assays. Methods. After 24 and 48 hours of incubation with a Fab(‘)3 anti-human IgD (10 µg/ml), unstimulated (US) and stimulated (S) CD19+ B cells isolated from unmutated CLL patients underwent microarray analysis using the HG-U133 Plus 2.0 Affymetrix arrays. Unsupervised clustering, t-test and Analysis of Variance (ANOVA) were performed. In addition, at 24 and 48 hours from the stimulus, antigenic expression was investigated by immunophenotypic analysis, cell cycle distribution changes were evaluated using the Acridine Orange (AO) technique, cell proliferation was measured by 3H-Tdr uptake and, finally, apoptosis was analyzed by the Annexin-V and/or AO technique. Results. Unsupervised gene expression analysis showed that all CLL cells were responsive to stimulation, regardless of the clinico-biological features. T-test performed between US and S samples confirmed these findings and allowed to identify 290 differentially expressed genes - mostly involved in BCR signaling, cell adhesion, antigen processing and presentation, and MAPK cascade - after 24 hours of stimulation. At variance, at 48 hours, we selected 188 transcripts involved in regulation of transcription, chromatin organization, apoptosis and cell differentiation, suggesting that in CLL cells gene expression activation following IgD cross-linking occurs at later time points of stimulation. Furthermore, to assess the effects of IgD ligation in specific CLL subgroups, we used two different supervised approaches: t-test and ANOVA. Both analyses showed that the IGHV configuration and the status of the disease of the cases evaluated did not affect the responsiveness of cells to BCR engagement via slgD. To validate the microarray results, we compared the antigen mean fluorescent intensity (MFI) ratio of US and S cases of a set of selected transcripts encoding for B-lineage antigens involved in cell activation. This approach confirmed the downmodulation of CD79a, CD79b, CD27 and CD62L in all samples upon IgD ligation. Next, at 24 and 48 hours from the stimulus, cell cycle analysis and proliferation assay documented that IgD cross-linking induced a decrease of proliferative activity in CLL cells, irrespective of their clinico-biological characteristics; accordingly, at the same time points apoptosis increased significantly in S samples. Conclusions. Gene expression profile highlights that the majority of CLL are responsive to IgD stimulation, irrespective of the clinico-biological characteristics of the samples analyzed. In agreement with microarray results, in vitro experiments have shown a reduction of cell proliferation and a concomitant increase in the apoptotic rate of S cases, providing new insights into the mechanisms that regulate BCR engagement via slgD in CLL.

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**CORTACTIN EXPRESSION IS TIGHTLY CONNECTED TO B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AGGRESSIVENESS**

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**Background.** B-cell Chronic Lymphocytic Leukemia (B-CLL) is a disorder characterized by the accumulation of clonal CD5+ B lympho-
cytes due to uncontrolled growth and resistance to apoptosis. Several protein kinase pathways have been claimed to be involved in the regulation of apoptosis and cell survival of the neoplastic clone. We previously demonstrated that Src kinase Lyn displays anomalous properties in leukemic cells when compared to normal B lymphocytes. Aims. The protein cortactin is an ubiquitous actin-binding protein; it was recently demonstrated to facilitate its interaction with signalling molecules such as PKC beta, β3/Raf-1, ERK and PKC beta following stimulation.

Methods. 130 CLL patients were treated in a single institution. We screened 35 patients and confirmed a correlation between cortactin expression and vimentin. Staining for vimentin was also performed in lymph node biopsies. Association of vimentin with Raf, pERK, 14-3-3 and PKC was assessed by immunoprecipitation and fluorescent microscopy. Results. We have screened 35 patients and confirmed a correlation between high vimentin levels and patients with poor prognostic markers (High CD58, Binet stage B/C, Poor risk cytogenetics and IGHV U4). Moreover in patients B-CLL lymphocytes (1.10±0.12 SE) with respect to normal B cells (0.19±0.06 SE, Student’s t-test p<0.05); when we investigated the correlation between level of cortactin and presence of somatic hypermutations (SHM) in the immunoglobulin heavy-chain variable region (IgVH) of B-CLL cells we found that unmutated patients expressed a higher level of cortactin (SHM+: 1.46±0.27 SE) with respect to mutated ones (SHM−: 0.90±0.11 SE, p<0.05, Student’s t test). We also evaluated the ability of neoplastic B cells to migrate after incubation with CXCL12/SDFlag chemokine and we observed that the overexpression of cortactin correlated with migratory activity of B cells. The expression level of cortactin was evaluated by real-time PCR, using GAPDH gene as calibrator, and by Western blotting analysis, using monoclonal Anti bodies against cortactin and β-actin as internal calibrator. Activity of matrix metalloproteinase 9 (MMP-9) in conditioned medium was evaluated by Zymography assay after 24h of culture with and without CXCL12/SDF1a (MMP-9) in conditioned medium was evaluated by Zymography assay after 24h of culture with and without CXCL12/SDF1a chemokine; expression level of MMP-9 was also evaluated by real-time PCR. Migratory activity of B cells was induced by CXCL12/SDFlag chemokine and we observed that the overexpression of cortactin was associated with an increased B-CLL migration index (r = 0.9). Finally, the release of MMP-9 in cultured medium by neoplastic cells was correlated with expression of cortactin measured by Zymography assay, patients without MMP-9 expression presented low cortactin level (0.46±0.20), while patients characterized by abundant expression of MMP-9 showed high cortactin level (2.43±0.04; p<0.05).

Conclusions. We found that cortactin is overexpressed in neoplastic B cells and that this overexpression correlated with migration index after CXCL12 stimulus and MMP-9 release in cultured medium, suggesting a role of cortactin in the aggressiveness of B-CLL neoplastic cells and support the cortactin as biomarker for diagnosis and prognosis of B-CLL.

**0655**

**NON-CANONICAL WNT SIGNALLING AND VIMENTIN IN CHRONIC LYMPHOCYTIC LEUKAEMIA (CLL)**

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**Background.** CLL is the most common adult leukaemia in the western world, with over 3000 new diagnosis per year in the UK. CLL represents a clonal population of B-lymphocytes characterized by low proliferation rates and defective apoptotic pathways. Although CLL is an extremely heterogeneous disorder there is a cohort of patients who require urgent treatment and usually have a rapidly fatal disease. This cohort have an aggressive disease and often exhibit poor prognostic markers (high CD58, Binet B/C, poor risk cytogenetics (11q−, 17p−)). These patients are not responsive to CLL within three years despite therapy, leading to over 1000 deaths per annum in the UK. Derogulation of Wnt signalling has been reported to occur in CLL, which may contribute to the pathogenesis of the disease through up regulation of vimentin. Vimentin is an intermediate filament, important for cell migration, signalling and determining the rigidity of lymphocytes. Moreover, expression of cortactin in a highly prognostic CLL population and the role Wnt signalling plays in regulating vimentin. Expression of vimentin, Wnts and Wnt signalling in B-CLL cell lines and CLL patient samples was assessed using RQ-PCR, Western blotting and flow cytometry. Staining for vimentin was also performed in lymph node biopsies. Association of vimentin with Raf, pERK, 14-3-3 and PKC was assessed by immunoprecipitation and fluorescent microscopy.

**Results.** We have screened 35 patients and confirmed a correlation between high vimentin levels and patients with poor prognostic markers (High CD58, Binet stage B/C, Poor risk cytogenetics and IGHV U4). Moreover in patients B-CLL lymphocytes (1.10±0.12 SE) with respect to normal B cells (0.19±0.06 SE, Student’s t-test p<0.05); when we investigated the correlation between level of cortactin and presence of somatic hypermutations (SHM) in the immunoglobulin heavy-chain variable region (IgVH) of B-CLL cells we found that unmutated patients expressed a higher level of cortactin (SHM+: 1.46±0.27 SE) with respect to mutated ones (SHM−: 0.90±0.11 SE, p<0.05, Student’s t test). We also evaluated the ability of neoplastic B cells to migrate after incubation with CXCL12/SDFlag chemokine and we observed that the overexpression of cortactin was associated with an increased B-CLL migration index (r = 0.9). Finally, the release of MMP-9 in cultured medium by neoplastic cells was correlated with expression of cortactin measured by Zymography assay, patients without MMP-9 expression presented low cortactin level (0.46±0.20), while patients characterized by abundant expression of MMP-9 showed high cortactin level (2.43±0.04; p<0.05).

**Conclusions.** We found that cortactin is overexpressed in neoplastic B cells and that this overexpression correlated with migration index after CXCL12 stimulus and MMP-9 release in cultured medium, suggesting a role of cortactin in the aggressiveness of B-CLL neoplastic cells and support the cortactin as biomarker for diagnosis and prognosis of B-CLL.
able (MRD+/not-CR) (p<0.001). Conclusions. Although studied in a heterogeneous cohort with a limit of detection of 2x10^-4, PB MRD status and clinical response were both found to significantly correlate to survival regardless of the line of treatment. Circulating residual B cells number and precise timing of the assessment did not seem to influence these results. PB MRD is clinically relevant in routine practice in CLL.

Figure 1.
THE IMPACT OF SPECIFIC MUTATIONS IN TP53 GENE ON THE RESULTS OF ALEMTUZUMAB THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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LEUKEMIA PATIENTS

RESULTS OF ALEMTUZUMAB THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA

The TP53 mutations located in the p53 DNA-binding motifs (DBM) were found in 37% of patients. The median age at diagnosis was 66 years (range 35 -91). Binet stage at presentation was as follows: stage A 191 (82%), stage B 23 (10%) and stage C 20 (8%). The median age was 66 years (range 35 -91). Binet stage at presentation was as follows: stage A 191 (82%), stage B 23 (10%) and stage C 20 (8%). The median duration of follow up was 54 months. The median monocyte count at presentation was 0.61 x10^9 /L (range 0.0 - 5.6 x10^9 /L). 74 (32%) patients presented with a monocyte count > 0.8 x10^9 /L which represents our laboratory upper limit of normal and Kaplan Meier analysis was used to assess TTF and OS for the entire cohort. Data were also analysed to exclude the confounding effects of known prognostic markers including Binet stage, CD38, IgVH mutational status and ZAP-70. Results. We identified 234 patients (M:F;150:84). The median age was 66 years (range 35 -91). Binet stage at presentation was as follows: stage A 191 (82%), stage B 23 (10%) and stage C 20 (8%). Mean duration of follow up was 54 months. The median monocyte count at presentation was 0.61 x10^9 /L (range 0.0 - 5.6 x10^9 /L). 74 (32%) patients presented with a monocyte count > 0.8 x10^9 /L of whom 45% commenced treatment outside the study. This did not achieve significance (95 vs 109 months, p=0.094). The prognostic importance of monocyte count was associated with Binet stage, but was independent of CD38, IgVH and ZAP-70. An elevated monocyte count was associated with a trend towards reduced OS, but this did not achieve significance (85 vs 109 months, p=0.094). Conclusions. Monocytosis at presentation in CLL is an independent adverse prognostic factor and is associated with a significantly shortened time to first treatment. This is a routinely and inexpensively measured parameter that warrants further investigation in prospective studies.
BCL2L12, A NOVEL MEMBER OF THE BCL2 FAMILY, IS DRAMATICALLY ELEVATED IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) PATIENTS AND CONSTITUTES AN UNFAVORABLE BIOMARKER IN CLL, PREDICTING POOR OVERALL SURVIVAL

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Background. BCL2L12 is a recently identified gene belonging to the BCL2 family, members of which are implicated in hematological malignancies, including chronic lymphocytic leukemia (CLL). Functional analysis of the novel cell survival-related BCL2L12 gene in patients with CLL, and to examine its prognostic and predictive value, and potential clinical application as a novel molecular biomarker for CLL. Methods. Total RNA was isolated from peripheral blood of 65 CLL patients and 23 healthy donors. An ultra-sensitive quantitative real-time PCR (qRT-PCR) methodology for BCL2L12 mRNA quantification was developed using SYBR® Green chemistry. After preparing cDNA by reverse transcription, relative quantification was performed using the comparative CT (2^(-ΔΔCt)) method. Moreover, analysis of IGHV mutational status, CD38 expression, and detection of apoptosis by double staining with annexin V-FITC and propidium iodide were performed. Results. BCL2L12 mRNA expression was significantly higher in CLL patients than in healthy donors (p<0.001). Moreover, ROC analysis demonstrated that BCL2L12 expression had significant discriminatory value, distinguishing very efficiently CLL patients from non-leukemic population (AUC=0.833, p<0.001). BCL2L12 expression was also shown to predict the presence of CLL, as demonstrated by both univariate and multivariate logistic regression analyses (p=0.001 and p=0.003, respectively). Finally, high BCL2L12 mRNA levels were associated with advanced clinical stage (p=0.028) and shorter overall survival (p=0.043) of CLL patients. Conclusions. BCL2L12 mRNA is overexpressed in the majority of CLL patients and constitutes a powerful predictor of the presence of the clinical course of the disease. In addition, high BCL2L12 mRNA expression is associated with advanced stage of the disease and predicts poor overall survival in CLL patients.

T REGULATORY CELLS (TREG) IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) TREATED WITH HIGH DOSE METHYPREDNISLONE (HDMP) AND RITUXIMAB (RTX)

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Background. Regulatory T cells were found to be lower in healthy population than in CLL patients. Treg were particularly increased in untreated CLL patients with advanced stage and higher Treg frequencies predicted shorter time to the first treatment. It has not been evaluated if Treg values predict survival in treated patients. Aim of the study was to evaluate Treg dynamics during treatment and their impact on survival. Methods. Two-center, single arm, open-label, prospective study was conducted to evaluate the efficacy of dose-dense HDMP in combination with Rtx in pretreated CLL patients with clinically or biologically high-risk disease. T regulatory cells, defined as CD4+CD25+CD127-FoxP3+ cells, were calculated as percentage of total CD4+ T lymphocytes and were assessed by 4-colour flow cytometry in peripheral blood at screening, after three treatment cycles and two months after the last treatment cycle. All patients provided informed consent. Results. 29 patients with CLL were enrolled. Median age was 59 years (range 45-76), 22 (76%) patients had Rai III-IV stage, 17 (59%) had bulky (>5 cm) lymphadenopathy. 25 (86%) patients had unmutated IgVH, 13 (45%) had 17p del and/or p53 mutation, 11 (38%) had 11q del, and one (3%) patient had trisomy 12, 10 (34%) were fludarabine refractory. Overall response rate (ORR) in 26 evaluable patients was 62%, all patients achieved partial response (PR). The median (range) Treg frequency at screening was 3.7% (0.06%-10.46%) and then decreased to 1.1% (0.007%-7.4%) after three treatment cycles and to 2.47% (0.2%-3.66%) at the end of therapy (p=0.001 and p=0.006 compared to Treg at screening, respectively). Treg frequency at screening did not correlate with response to treatment (p = 0.661) or with other prognostic factors such as bulky lymphadenopathy (p = 0.829), fludarabine refractoriness (p = 0.109), adverse cytogenetics (p = 0.676). The median follow-up for all patients was 22 months (range: 1-57). The median progression-free survival (PFS) was 12 months (95% CI: 8-16) and the median overall survival (OS) was 31 months (95% CI: 20-42). More prominent Treg decrease between screening and 3 treatment cycles and between screening and the end of therapy predicted better PFS (p = 0.036 and p=0.041, respectively) by linear regression method. In univariate analysis, patients with higher Treg frequency reduction between screening and three treatment cycles had a trend towards better OS (p=0.084) in multivariate analysis, only response to treatment (p=0.01) had a significant impact on PFS and fludarabine refractoriness (p=0.022) and response to treatment (p=0.01) predicted OS. Conclusion. HDMP and Rtx combination effectively reduces T regulatory cells in pretreated high risk CLL patients. However, Treg reduction during treatment was not an independent predictive factor for either PFS or OS. Further evaluation of T lymphocyte subsets and their impact on survival is being performed. (ClinicalTrials.gov identifier: NCT008 58181; supported by EEA and Norway grant No. 2004-LT0040-IP-1EEE).
of bone marrow infiltration. 3 were unmutated, no patient presented 17p-, 1 had 11q- and 7/9 had ZAP70 (+). The only correlation of borderline significance was between ZAP (+) and TCL (+). Among 24 SMZL patients with median age 61 years (45-78), 10 were male, 10 had elevated LDH and 3 patients were classified as high/intermediate IPI. ZAP70 and TCL1 were evaluated in 21 patients. 20/21 were negative and only one was found positive to both antigens. Conclusions. Immunohistochemical detection of TCL1 and ZAP70 is feasible in CLL, TCL1 (+) and ZAP70 (-) correlation is statistically significant in CLL patients. A TCL1 positivity may be associated with other unfavorable CLL markers such as BM infiltration pattern and ALC. Expression of TCL1 and ZAP70 is rare in SMZL patients.

LIPOPROTEIN LIASE AS A PROGNOSTIC MARKER AND IGHV MUTATION SURROGATE IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Chronic lymphocytic leukemia (CLL) is a highly heterogeneous condition clinically and biologically. In the era of rapidly improving treatment options, there is an increasing demand to predict outcome early in the course of the disease, a task not fulfilled by clinical stage. Among the numerous markers introduced recently, lipoprotein lipase (LPL) has been reported to be informative as a prognostic marker, related closely to IgVH mutation status in CLL. Aims. Our objective was to test LPL in the clinical practice as a predictor of outcome and correlate its expression with other prognosticators. Methods. Using peripheral blood samples of 73 unselected CLL patients, lipoprotein lipase mRNA levels were determined by quantitative PCR. LPL/ABL expression levels were normalized to healthy controls. A cutoff of 1.77 was established by ROC analysis in order to distinguish between positive and negative clones. IgVH mutation status was analysed by a multiplex PCR method and BIOMED-2 standardized primers. Greater than 4% sequence divergence from the germline was considered mutated. For CDS8 evaluation a 7% cutoff was applied. Results. LPL positive patients showed significantly shorter survival than negative ones (median survival 175 months vs not reached, p<0.0001). A close relationship was found between LPL expression and IgVH mutation status with 22 of 24 (92%) LPL positive cases being unmaturated and 23/32 (72%) LPL negative cases mutated. When CDS8 was employed as an IgVH surrogate, the sensitivity and specificity were 78% and 68%, respectively. A linear relationship was detected between LPL and CDS8 (p<0.005). Interestingly, within the good prognostic group having 13q-, LPL positivity identified a subgroup with shorter survival (median survival 136 months vs not reached, p<0.009). Conclusions. As measured by PCR from unselected peripheral blood, LPL determination proved to be a powerful predictor of outcome refining prognosis in the good-risk category of CLL. As an IgVH mutation surrogate, LPL outperformed CDS8 in this series. These findings justify further study and eventually, the routine application of LPL in the diagnostics of CLL.

HUMAN AND MOUSE DOCK10 SPlicing ISOFORMS WITH ALTERNATIVE FIRST CODING EXON USAge DIFFERENTIALLY EXPRESSED IN T AND B LYMPHOCYTES

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Background. Dedicator of cytokinesis (DOCK) proteins are a family of guanosine nucleotide exchange factors (GEF) for Rho GTPases, constituted by 11 mammalian members. Diverse Rho GTPases play crucial roles in lymphocyte development, activation, differentiation and migration. We have previously reported the cloning of a full length human DOCK10 cDNA [Yelo et al, Mol Immunol 2008; 45: 3411-8], whose expression is distinctively induced by IL-4 in normal peripheral blood B lymphocytes and chronic lymphocytic leukemia (CLL) B cells. Here, we analyzed DOCK10 mRNA diversity produced as a result of alternative splicing. Aims. To define the DOCK10 protein-coding transcripts in the human and the mouse and to gain insights into regulation of their expression. Methods. Full-length cDNA clones of DOCK10 were obtained from total RNA of both normal human peripheral blood mononuclear cells (PBMC) (10 clones from 10 individuals, total 100 clones) and mouse spleen (10 clones), by high fidelity RT-PCR. B and T lymphocytes from normal individuals and CLL B cells were isolated using negative selection and cultured in the presence of 10 ng/ml of IL4 (BD Pharmingen). Expression of the DOCK10 isoforms was measured by quantitative PCR (Q-PCR) and western blotting. Panels of human and mouse tissues were analyzed for mRNA expression. Protein and mRNA expression were also studied in a panel of human lymphoid, myeloid and epithelial cell lines. Results. Alternative first coding exon usage led to two main protein-coding transcripts, which we named DOCK10.1 and DOCK10.2. Full-length cDNA clones of both isoforms were obtained from both normal human PBMC and mouse spleen, for the first time for human DOCK10.1, mouse DOCK10.1, and mouse DOCK10.2. Human and mouse DOCK10.1 clones corresponded to the protein coding assemblies provided by NCBI as Reference Sequences (RefSeq) for DOCK10. Our analysis especially focused on human cDNA clones, of which 63% were alternatively spliced forms involving diverse exons and introns (17% in frame, 46% frame shifts). The most frequent variations were shortened versions of exons 5, 12, 18, 35, 45, and 45. Deletions of entire exons were also found, e.g., exons 8, 28, 31, and 40. The consensus rules for intron-exon boundaries were fulfilled in all the subvariants. DOCK10.1 expression was enriched in normal T cells, and DOCK10.2 expression was enriched in normal and CLL B cells. Some lymphoid cell lines express one isoform preferentially over the other. Both isoforms were up-regulated in response to interferon-4 (IFN-4) in B cells, both normal and CLL, but not in T cells. Summary/Conclusions. Two DOCK10 isoforms, named DOCK10.1 and DOCK10.2, arise from the use of alternative first exons. Whether the frequent shortened and truncated isoforms play specific functional roles or are just transcriptional noise remains to be elucidated. The two isoforms are expressed in human and mouse tissues, mainly in lymphocytes and lymphocyte-rich organs. DOCK10.1 is enriched in T cells and DOCK10.2 in B cells. IL4-treatment up-regulates both isoforms in B cells, but not in T cells. Our data suggest that cell-specific mechanisms regulate expression of the alternative first exon variants of DOCK10 in vertebrates. antonio.parrado@carm.es
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HAPLOTYPE FOR POLYMORPHISMS C1236T, C3435T AND G2677T/A IN MDR1GENE IS ASSOCIATED WITH MAJOR MOLECULAR RESPONSE IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH STANDARD-DOSE OF IMATINIB

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Background. Chronic myeloid leukemia (CML) is a clonal expansion of hematopoietic progenitor cells, resulting in myeloid hyperplasia, leukocytosis, neutrophilia, basophilia and splenomegaly. Imatinib Mesylate (IM) used in the treatment of CML, interacts with membrane efflux transporters such as MDR1. Polymorphisms in this gene have been established by European Leukemia Net. Patients with cytogenetic patterns established were studied. All patients were initially treated with a standard dose of IM (400 mg/day) and divided in two groups according to response. The 1st group ("responder") comprised 70 patients who had a complete cytogenetic response within 18 months of treatment. The 2nd group ("non responder") comprised 48 patients who did not have a complete cytogenetic response with the initial dose (400 mg/day) of IM or who relapsed during treatment and were submitted to higher doses of 600 or 800 mg/day. Criteria of failed response to treatment were established by European Leukemia Net. Patients with cytogenetic patterns other than the Philadelphia chromosome and patients with mutations in the BCR-ABL1 gene were excluded from this study. Major molecular response (MMR) was defined as a reduction of BCR-ABL1 transcripts in the peripheral blood standardized on the International scale. MDR1 gene polymorphisms were determined on all patients using RFLP PCR. Results. The frequencies of MDR1 variant alleles were 41.1% (1236T), 37.3% (3435T), 32.2% (2677T) and 2.1% (2677A), respectively for C1236T, C3435T, G2677T/A polymorphisms. No subject had the MDR1 2677AA genotype. The genotypes frequencies for C1236T, C3435T and G2677T/A polymorphisms were similar in the two groups (responder and non responder; p>0.05). In the responder group, the frequency of 1236CT/2677GT/3435CT haplotype was higher in patients with MMR (51.7%) than in patients without MMR (8.3%, p=0.010). Furthermore, carriers of 1236CT/2677GT/3435CT haplotype had a 11.8 fold greater odds ratio (95%CI: 1.43-97.3, p=0.022) of achieving molecular response compared with all who had others haplotypes in logistic regression. Conclusions. The 1236CT/2677GT/3435CT haplotype in the MDR1 gene is positively associated with major molecular response to treatment with IM.

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CONNECTING DIAGNOSTIC RISK SCORES TO INDIVIDUAL PATIENT RESPONSE UNDER NILOTINIB THERAPY

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Introduction. At diagnosis early chronic phase CML (ECP-CML) patients are typically categorized in low, intermediate or high-risk groups using the Sokal or Hasford scoring systems, that provide an indication of the patient survival probability if treated with chemotherapy or interferon respectively. Given that tyrosine-kinase inhibitors have become the first-line treatment method for these patients, one can wonder how individual patient response to drugs like imatinib or nilotinib relates to the standard scoring systems at diagnosis. Methods. Combining a computational model of hematopoiesis and CML with individual serial Q-RT-PCR data for disease burden (BCR-ABL) under nilotinib therapy of 75 patients, we estimated for every patient independently the self-renewal probability of CML cells (eCML), the fraction of CML cells responding to therapy (eNILO) and the impact of TKI on the self-renewal probability of CML cells (eNILO) under therapy. These estimates were obtained from the data on response to treatment, and independent of the calculation of the prognostic category. Results. The results show that two model features, i.e. self-renewal probabilities for CML cells (eCML) and treated CML cells (eNILO), provide the best partitioning of the response data. The first feature differentiates between two clusters: One cluster (A) with eCML<0.7 and another (B) with eCML>0.7, that partition patients into two clusters. Within each group, the second feature differentiates between patients responding optimally or suboptimally to therapy, making it possible to introduce again two types of subclusters. Examining the relationship between the cluster a patient belongs to based on response dynamics and its Sokal or Hasford score shows only a slight correlation: Most low-risk patients are in cluster A, whereas most high-risk are in cluster B. However patients from all prognostic categories can be found in most clusters and those in the best group are not necessarily patients in the low-risk Sokal group. Conclusions. Through our computational model of hematopoiesis and CML we can classify patients' response to therapy based on two important features: the fitness of the CML cell before therapy and the fitness-change induced by treatment. Together these features allow one to categorize a patient response to TKI treatment and correlate it with prognostic scores calculated at diagnosis. Our results show that the correlation appears to be weak, requiring on the one hand that new prognostic tools based on TKI therapy should be designed and on the other hand that one might take early therapy-response markers into account to improve patient treatment in the long run.

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THE GENE EXPRESSION EVALUATION OF THE SEPT5 IN PATIENTS WITH CML, AT THE DIAGNOSIS, HEALTHY BLOOD DONORS AND CELL LINES OF HUMAN AND MURINE

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Background. SEPT5 is a member of nucleotide binding proteins, called septins that were firstly described in yeast as cell division cycle regulatory proteins. This gene was reported in patients with acute myeloid
leukemia translocated with MLL gene and with high expression in myelodysplastic leukemia cell line and in adult human bone and heart in association with alpha granules of human blood platelets. In a recent study using SSH libraries, we compared the gene expression pattern between granulocytes of health control and CML patients, and we identified this gene expressed only in CML patients. Although several studies in literature, there is not a clear relationship between the expression of this gene and the development and progression of CML. Methods and Patients. The evaluation was made in peripheral blood granulocytes and mononuclear cells of CML patients and healthy blood donors, and in the cell lines K562, BaF3/BCR-ABLp210 and BaF3T315I using real time PCR. Results. To validate the result found in the SSH library, the gene expression of SEPT5 was evaluated by real time PCR using the same samples used in the library construction. These results confirmed our previous results showing that the SEPT5 expression is increased in the granulocytes of CML patients. The same results was observed when we studied the expression comparing individually 11 patients at diagnosis and 05 health controls, suggesting that this protein could be increased in all human lines that have the translocation BCR-ABL. However, using cell lines of murine BaF3/BCR-ABLp210 and BaF3T315I, the expression of this gene was absent, suggesting that this gene had a very low expression in the translocated cells of this model and that could be involved in the human CML. Conclusion. Despite major advances in the treatment of CML, the treatments available are not capable of inactivating all the pathways activated by BCR / ABL. Our results demonstrate that SEPT5 could be involved in the development of CML and the importance of the study of possible pathways that could culminate in its high expression or the triggering of other unknown pathways involved in the development of CML by this gene. This work was supported by FAPESP.


Background. The t(9;22)(q34;q11) translocation, resulting in the BCR-ABL1 fusion gene and subsequent constitutively activated tyrosine kinase is both the hallmark and initiator of Chronic Myeloid Leukaemia (CML). Although initially indolent and generally responsive to first line tyrosine kinase inhibitor therapy (Imatinib), heterogeneity of response is observed in a proportion of patients, leading to primary and secondary resistance, suggesting that other genetic lesions play a role in determining outcome. Aurora Kinase A and B (AURKA and AURKB) have roles linked to oncogenesis (OfOf) and their dysregulation is frequently observed in solid and liquid cancers and is associated with chromosomal instability. The MGMT gene encodes a DNA repair protein and its expression is known to be epigenetically associated with chromosomal instability. Down-regulation of MGMT plays a critical role in maintaining genomic stability. Down-regulation of MGMT expression is a marker of increased risk of treatment failure and is associated with a poor outcome. Aims. To ascertain whether the differential expression of AURKA, AURKB and MGMT correlated to treatment response, and additionally to investigate whether promoter CpG methylation played a role in MGMT down-regulation. Methods. Gene expression of AURKA, AURKB and MGMT in CML cell lines K562, BaF3/BCR-ABLp210 and BaF3T315I was measured by RT-qPCR. Results. The expression of AURKA and AURKB was significantly up-regulated (p < 0.05) in BaF3T315I compared to K562, but not in BaF3/BCR-ABLp210. MGMT expression was significantly up-regulated in BaF3T315I compared to K562 and BaF3/BCR-ABLp210 (p < 0.01). The expression levels of AURKA and AURKB were 5-10 fold higher in BaF3T315I than in BaF3/BCR-ABLp210 and K562. Furthermore, the expression of AURKA and AURKB were significantly up-regulated in CCR compared to those with lower expression (p < 0.001). Conclusion. The expression of AURKA and AURKB is not affected by promoter CpG methylation, whereas MGMT expression is down-regulated in CCR.
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DIFFERENTIAL ROLE OF THE JAK2-STAT5 SIGNALING MODULE IN BCR-ABL-DEPENDENT TRANSFORMATION
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Background. Activation of the transcription factor STAT5 is a critical event for the initiation and maintenance of Chronic Myeloid Leukemia (CML). Bcr-Abl is linked to the activation of the tyrosine kinase Bcr-Ab1. Aim. A possibility to inhibit STAT5 activation in CML could be the use tyrosine kinase inhibitors (TKIs) targeting the JAK2 kinase that was shown to be the upstream activator of STAT5 in different physiological and pathological setting. Methods/Results: Using conditional JAK2 mouse knock-out models, we show that JAK2 is dispensable for myeloid and lymphoid Bcr-Ab1-driven leukemia and initial myeloid transformation, whereas JAK2 is required for initial lymphoid transformation. These observations speak for an uncoupling of STAT5 activation from JAK2 at different stages of transformation depending on the oncprotein. We further show that JAK2 TKIs induce apoptosis in JAK2-deficient cells irrespective of the presence of JAK2. This is likely caused by the direct 'off-target' inhibition of Bcr-Ab1. Using a combination of siRNA and pharmacological interference excluded a requirement for tyrosine kinases other than Bcr-Ab1 for STAT5 activation and indicating a direct phosphorylation of STAT5 by Bcr-Ab1. This finding was further supported by enzymatic studies that indicated STAT5 phosphorylation with similar efficiency than the known Bcr-Ab1 substrate CrkL. Summary. Our results excluded a dominant role of JAK2 in Bcr-Ab1 dependent transformation and withdraw the rationale of a clinical use of JAK2 TKIs in CML.

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HIGH-RESOLUTION MELTING CURVE ANALYSIS FOR SCREENING FOR MUTATIONS IN THE BCR-ABL KINASE DOMAIN OF CML PATIENTS
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Background. Chronic myeloid leukaemia (CML) is characterized by the BCR-ABL fusion oncogene. The oncogene encodes for a tyrosine kinase that can effectively be inhibited by tyrosine kinase inhibitors (TKIs). Nevertheless, some patients acquire resistance to treatment as a result of mutations in the BCR-ABL kinase domain. Mutations impair the binding of TKIs to the kinase domain. Thus the detection of mutations in the kinase domain is important to explain sub-optimally response to TKIs. Mutations in the BCR-ABL kinase domain are usually detected through sequencing. Recently, high-resolution melting curve analysis (HRM) has been suggested as a method to screen the BCR-ABL kinase domain for single base mutations prior to sequencing. There have also been a few reports on the possible impact of indels in the kinase domain and gene amplification on patient prognosis. However it is not known whether HRM can be used to identify indels or duplications associated with the kinase domain. Aim. The aim of this study was to determine whether HRM analysis can be used to screen the BCR-ABL kinase domain for mutations including indels and duplications prior to sequencing. Methods. A study was obtained from 33 CML patients on TKI treatment. Of these, 13 patients were suspected of having mutations due to sub-optimal response or a loss of response with TKIs. Sequencing of the kinase domain was performed according to Branford and Hughes (2006). HRM analysis was performed using the Melt Doctor HRM Reagent Kit and primers from Poláková et al. (2008) on the ABI 7500 Fast. Results. Thirteen samples were found to contain mutations in the BCR-ABL kinase domain, including three samples with single base changes, one with a previously described 35 base pair insertion and nine with duplications of which seven contained indels not previously described. There was an overlap in 1m range for samples with mutations compared to samples without mutations, irrespective of the mutation type and so difference plots were used to identify samples with mutations. Samples with duplications were identified as different variants in the difference plot, from those with only single base mutations. Compared to this, samples with either one or two single base mutations were found in the same variant group in the difference plot. Conclusions. HRM analysis was successfully used to screen for mutations in the BCR-ABL kinase domain. Samples with mutations were not readily identifiable from shifts in Tm but were indicated as variants in the difference plot. An insertion in the BCR-ABL kinase domain as well as duplications of the kinase domain, were identified by HRM analysis. Based on this study, HRM analysis can be used to screen the kinase domain for mutations including single base changes, indels and duplications, prior to sequencing.

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IDENTIFICATION OF SARI (SUPPRESSOR OF AP-1, REGULATED BY IFN) DOWN-REGULATED BY BCR-ABL IN K562 CELLS: A NOVEL TARGET FOR TREATMENT OF CML
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Backgrounds. SARI (Suppressor of AP-1, Regulated by IFN) is a novel basic leucine zipper containing type I IFN-inducible early response gene that exerts cancer-selective growth inhibitory effects. The constitutive SARI expression was detected in multiple lineage-specific normal cells, whereas its expression was not detected in their tumorigenic counterparts. However, little is known so far about SARI expression in patients with CML (chronic myelogenous leukemia), the regulation of SARI expression in CML cells. Aim. To investigate whether Bcr-Ab1 would play a role in down-regulation of SARI expression in CML-derived cell line K562. Methods. Forty-six Chinese patients with CML and forty healthy Chinese volunteers were recruited and informed consent at Union hospital of Wuhan in this study. SARI expression in the peripheral blood mononuclear cell of CML patients and healthy Chinese volunteers was assayed by using Real-time quantitative PCR. In vitro, in respective experiment, K562 cells were incubated with the Bcr-Ab1 inhibitor STI571 (Imatinib, Gleevec) (0.5, 1.5, 2.5µM) at 37°C and 5% CO2 for 6, 12, 24-hours followed by detection of SARI expression using Real-time quantitative PCR. Further, the correlative downstream pathways were identified by using signal pathway inhibitors. K562 cells were incubated with PI3-kinase inhibitor LY294002 (20µM), MEK inhibitor PD98, 059 (50µM), JAK inhibitor Ag490 (50µM) at 37°C and 5% CO2 for 24 hours in respective experiment, then SARI expression were detected by Real-time PCR. All experiments were repeated three times. Statistical analysis was performed using SPSS 17.0. Results. Compared with healthy volunteers, expression of SARI mRNA in PBMCs of CML patients presented a lower level (p<0.001). In vitro, after exposure of K562 cells to STI571, the SARI expression was higher than those in control K562 cells (without STI571 treatment). SARI expression was enhanced in K562 cells as early as 6hours after treated with STI571 (1.5µM) and SARI expression was up-regulated in a time-dependent manner. In addition, after treated with JAK inhibitor Ag490, PI3K inhibitor LY294002 and MEK inhibitor PD98, 059 respectively, SARI mRNA expression was up-regulated by Ag490 (p<0.05) and PD98, 059 (p<0.001), but not by LY294002 compared with control K562 cells (without signal treatment). Conclusion. The SARI down-regulation is implicated in CML pathogenesis. Bcr-Ab1 mediates the down-regulation of SARI mRNA expression in K562 cells. Moreover, Bcl-Ab1 down-stream signal pathways, including JAK signal pathway and MEK signal pathway are involved in the down-regulation of SARI mRNA expression in K562 cells. These findings suggest that SARI is a potential gene target in CML therapy.

0674
THE ROLE OF CELL DIVISION CYCLE 6 OVEREXPRESSION IN CHRONIC MYELOID LEUKEMIA CELLS
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Background. The cell division cycle 6 (Cdc6), a protein in eukaryotic cells, is an essential regulator of DNA replication and plays important roles in the activation and maintenance of the checkpoint mechanisms in the cell cycle that coordinate S phase and mitosis. Recent documents indicated the deregulation of Cdc6 expression in human cell proliferation and in a serious range of carcinogenesis. Down-regulation of Cdc6 was observed in prostate cancer. Up-regulation of Cdc6 was found in cervical cancer, lung cancer and brain cancer. Chronic myeloid leukemia (CML) is a form of leukemia characterized by the
increased and unregulated growth of predominantly myeloid cells in the bone marrow and the accumulation of these cells in the blood. And the cause of CML was confirmed the central importance of BCR-ABL. But up to date, the relationship of Cdc 6 and CML is still obscure. Aims. Our Aim is to investigate the expression of Cdc6 and its role in CML. Methods. The expression of Cdc6 in normal bone marrow mononuclear cells, CML primary cells and K562 cells (CML cell line) was detected by real time quantitative RT-PCR and immunofluorescence assay. The effects of Cdc6 gene silencing by siRNA on cell proliferation and apoptosis in K562 cells were evaluated by CCK-8 assay and Flow Cytometry. The effects of specific inhibitors Imatinib, LY294002, PD98059 and AG490, which separately target BCR/ABL, PI3K, MAPKK and JAK, on the expression of Cdc6 in K562 cells were determined by real time quantitative RT-PCR and Western Blot. Results. Cdc6 expression was significantly up-regulated in CML primary cells and K562 cells, compared to normal bone marrow mononuclear cells. Cdc6 gene silencing by siRNA effectively inhibited DNA replication and induced apoptosis in K562 cells. Imatinib, a BCR/ABL inhibitor, induced down-regulation of Cdc6 expression in K562 cells. LY294002 and AG490, but not PD98059, decreased the expression of Cdc6 in K562. Conclusions. These data indicated Cdc6 overexpression contributed to the high proliferative activity and the low apoptosis, and regulated by BCR-ABL signal transduction through downstream PI3K/Akt and JAK/STAT pathways in CML cells. It was also suggested that Cdc6 protein may be an attractive target for the development of effective anti-cancer strategies in adult chronic myeloid leukemia patients.

0675 EXPRESSION OF TRANSKETOLASE-LIKE GENE 1 (TKTL1) CHANGES DURING ACCELERATION OF CHRONIC MYELOID LEUKEMIA

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Background. Development of resistance or progression to blast phase in chronic myeloid leukemia (CML) occurs in a significant proportion of patients under tyrosine kinase inhibitor (TKI) therapy. Overexpression of transketolase-like gene 1 (TKTL-1) on mRNA and protein level has been linked to tumour progression, metastasis and poor patient outcome in many solid tumours. A TKTL1 knockdown in tumour cells resulted in reduced activity of the pentose phosphate pathway (PPP), lower lactate production and G0/G1 arrest. Therefore TKTL1 is regarded as a potential target for drug therapy. Until today little is known about TKTL1 expression in CML. Aims. We sought to evaluate TKTL1 gene expression in different CML phases. Patients and Methods. 108 peripheral blood samples of 79 CML patients (pts) (median age 56 years, range from 17 to 84) were investigated. 49 samples were collected from pts in chronic phase (CP), 22 from pts in major molecular response (MMR), 22 from accelerated phase (AP) and 15 in blast crisis (BC) pts. A control group consisted of 21 healthy individuals. TKTL1 mRNA expression levels were determined by quantitative reverse transcription PCR (qRT-PCR) using LightCycler® technology and normalized against 18S rRNA. TKTL1 expression has also been determined in granulocytes isolated by Ficoll gradient centrifugation from blood and in immature CD34+ and CD34+/CD33+ cells isolated from bone marrow by MACS beads technology. Results. A significantly lower TKTL1 expression was found in the CP group compared to the group of healthy donors (TKTL1/GUS ratio 2.5% vs 8.7%, p<0.01, Table). Intermediate expression levels were observed in AP (1.1%). Lowest expression levels were observed in the BC group (0.9%). No significant difference could be found in the MMR vs baseline (6.4% vs 8.7%, p=0.25). In addition, this evaluation revealed no significant difference of TKTL1 expression at baseline in CP patients with subsequent favourable outcome (MMR within one year) and those with subsequent progression (AE or BC) during therapy. Further experiments showed significantly higher TKTL1 expression in mature granulocytes in comparison to immature CD34+ and CD34-/CD33+ cells. Conclusions. TKTL1 expression levels appear to decline in the course of CML with lowest levels during BC. This might be due to the suppression of matured cells by the clone of immature blasts with a lower expression of TKTL1. However, in this study TKTL1 expression levels at diagnosis failed to give a predictive information about disease progression.

0676 IMATINIB DISCONTINUATION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA WHO HAVE RECEIVED FRONT-LINE IMATINIB THERAPY AND ACHIEVED COMPLETE MOLECULAR RESPONSE

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Background. Imatinib (IM) is an effective treatment in patients with chronic myeloid leukemia (CML). Complete molecular response (CMR) was only attained in 10-20% of chronic phase (CP) CML patients at 24 months, and the proportion was substantially increased by continuing IM therapy. Therefore we evaluated the complete molecular response rate (CMR) for 2 years after IM therapy in patients with chronic myeloid leukemia (CML) patients, who had their IM therapy discontinued after achieving CMR. Methods. We consecutively enrolled CP CML patients, who had their IM therapy discontinued after achieving CMR during at least one year on IM therapy at 2 Korean institutions from September 1999 to September 2008. We compared the measurable levels of BCR-ABL transcript by RQ-PCR with at least 10,000 ABL transcripts per volume cDNA. After discontinuation, BCR-ABL/ABL ratio was monitored by RQ-PCR monthly during the first 6 months and every 3 months thereafter, and relapse was defined by detectable levels of BCR-ABL transcript in two successive assays. Results. Fourteen patients were included. The median patient age was 58 years, and 10 patients were women. All patients were CP and received IM as an initial treatment. Sokal score at diagnosis was low in 1 patient, intermediate in 6, and high in 7. IM was started at a dose of 400mg/day. The median interval from IM initiation to CMR was 19.2 (range, 7.0-71.9) months. After achieving CMR, IM therapy was continued for a median of 32.3 (range, 12.1-72.4) months. And, the median duration of IM therapy was 56.4 (26.2-82.0) months. After IM discontinuation, molecular relapse occurred in 9 patients at 1.1 to 6.9 months. With a median follow-up of 13.4 (2.5-20.4) months, TTLC at 1-year was 31.4% (95% CI, 15.5-44.5). In the univariate analysis of factors affecting molecular relapse, high-risk of Sokal score (p=0.004) and more than 24 months of interval between IM initiation and CMR (p=0.008) were associated with frequent molecular relapse. Duration of IM after CMR was not significantly related with molecular relapse, but there was a trend of lower molecular relapse in patients with at least 24 months after achieving CMR. IM was resumed in all patients with molecular relapse. 6 of 9 patients showed decrease in their BCR-ABL transcript levels and 3 achieved a CMR after IM rechallenge. Conclusion. IM discontinuation in patients with CML who have received IM as initial treatment and achieved a CMR might be feasible, as one-third of patients remain CMR after at least one year of IM therapy. However, continued treatment with IM might be recommended after IM rechallenge.
Bcr-Abi Mutations in Imatinib Resistant Patients with CML and Impact of Second Generation Kinase Inhibitors on Baseline Mutations Treatment - A Study on Behalf of Latin American Leukemia Net (LALNET)

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Background. Mutations within the BCR-ABL domain are the most frequent mechanism of imatinib (IM) resistance. The second generation kinase inhibitors (SGI) are indicated for imatinib intolerance or resistance and the initial trials showed similar response rates in IM resistant patients after IM failure, independent of mutation status, except T315I. Aims. The aim of this work was to report the frequency of BCR-ABL mutations in chronic myeloid leukemia (CML) patients with imatinib resistance in a Latin American population and to evaluate the clinical impact of SGI treatment in this group of patients. Methods. A total of 187 resistant patients with BCR-ABL mutations were analyzed. After imatinib resistance, 120 received IM (81 dasatinib, 28 nilotinib and 5 bosutinib). Mutations were detected by direct sequencing from bone marrow or peripheral blood samples, collected during imatinib (129), dasatinib (29), nilotinib (21) or other treatments (8). The median follow-up from mutation detection was 12 months. Overall survival (OS) was calculated from date of mutation detection until last follow-up or death, whereas the progression-free survival (PFS) from date of mutation detection until progression to accelerated phase or blast crisis, last follow-up or death. For the statistical analysis was used log-rank test using SPSS 14.0 software. Results. The median age of patients at diagnosis was 42 years. 75% were in CP, 19% in AP and 8% in BC. According to Sokal score, patients were stratified in low risk (27%), intermediate (36%) and high (37%); 32% had used Interferon. The median follow-up from mutation detection until progression to accelerated phase or blast crisis, last follow-up or death, was 12 months (6-106). Considering all patients, the mean and median of adherence were 89% and 96% respectively and 24% of patients were completely adherents with 100% of MFR. Adherence decreased with longer term therapy (p=0.02) and longer duration of disease (p=0.002). The adherence was superior in patients enrolled in clinical trials (p=0.007). CMR rates were superior in patients more adherent (p=0.01 - ANOVA and p=0.02 - Bonferroni). On the other hand, adherence was inferior in patients using higher dose (800/800mg) of IM (p=0.01). There was not significant difference in adherence regarding socioeconomic status, marital status, Sokal and institutional level. Conclusion. Higher adherence in CML patients using IM is related with superior CMR, IM lower doses and clinical trial participating. However, the poor compliance was associated with longer term therapy and longer duration of disease.
erature. Uncertainty was assessed using intervals comprising 95% of 1,000 model replications generated via Monte-Carlo simulations (95% credible intervals [CI]). Results. Initiating TKI therapy with nilotinib resulted in an estimated mean (95% CI) LE of 28.33 (26.73-29.94) years and quality-adjusted LE of 25.14 (23.42-26.73) years. The corresponding estimates for imatinib first-line therapy were 24.43 (22.70-25.97) years and 21.56 (19.87-23.33) years, respectively. The differences in mean (95% CI) quality-adjusted LE between nilotinib and imatinib were 3.68 (2.04-5.35) years and 3.58 (2.03-5.14) years, respectively. Conclusions. First-line nilotinib was more efficacious than imatinib in the ENESTnd trial at 12 months. As trial data collection is ongoing, modeling can be used to estimate the long-term value of these treatments. The present model suggests that nilotinib is likely to substantially increase quality-adjusted LE v. imatinib.

0680 IMPROVEMENT OF IMATINIB-RELATED CHRONIC LOW-GRADE NONHEMATOLOGIC ADVERSE EVENTS (AES) IN PHILADELPHIA-POSITIVE (Ph+) CHRONIC MYELOID LEUKEMIA-CHRONIC PHASE (CML-CP) PATIENTS SWITCHED TO NILOTINIB

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Methods. A total of 846 patients were randomized to receive nilotinib 300 mg BID (n=282), nilotinib 400 mg BID (n=281), or imatinib 400 mg daily for ≥2 months or recurring more than 3 times and persisting despite best supportive care. At enrollment, patients are switched to nilotinib 300 mg twice daily. The primary endpoint is change in imatinib-related nonhematologic AEs using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 after 3 months (end of cycle [EOC] 3) of nilotinib. Secondary endpoints include response to nilotinib and patient-reported outcomes as measured by QOL questionnaire and the MD Anderson Symptom Inventory (MDASI)-CML. The study was conducted in accordance with Declaration of Helsinki; patients provided written informed consent. This preliminary analysis was performed on 23 patients enrolled as of the data cut-off date of November 30, 2010. Results. The median duration of nilotinib was 3.6 months. There were 105 baseline imatinib-related nonhematologic AEs (72 Grade 1; 33 Grade 2) persisting for a median 24.8 months. Fifteen patients completed EOC 3 by cut-off. Of the 75 imatinib-related AEs reported among these patients, 45 AEs were improved (30, 7, and 3 AEs resolved by months 1, 2, 3, respectively; 5 AEs decreased from Grade 2 to 1); 27 were unchanged, and 3 increased in severity. At study entry, 20 patients had achieved major molecular response (MMR, 3-log reduction of Bcr-ABL); 3 additional patients achieved MMR by cut-off. Eleven patients completed QOL questionnaires; 82% reported QOL improvements at EOC 3 compared to baseline. Mean reduction from baseline in MDASI-CML severity score and interference score were 1.47 & 1.86 (EOC 1) and 1.45 & 1.53 (EOC 3), respectively, indicating improvement in symptoms. Nilotinib dose was reduced in 7 patients for nilotinib-related nonhematologic AEs. Fourteen Grade 3 AEs were reported in 6 patients, 10 increased bilirubin and lipase, dehydration, hypokalemia, hypophosphatemia, worsening of arthralgia, joint pain) were suspected to be nilotinib-related. No patient had a Grade 4 AE. Most AEs were managed by brief dose interruption. Two patients discontinued nilotinib (1 hyperglycemia, 1 upper abdominal pain/oral pain/headache). The maximum QTcF change from baseline was 57 msec; QTcF prolongation >500 msec did not occur. Conclusions. Switching to nilotinib improved nonhematologic imatinib-related, low-grade AEs in >50% of CML-CP patients in this analysis. Overall improvement in symptoms was observed and 82% of patients experienced improved QOL, including 8 of 6 patients with Grade 3 AEs. Long-term evaluation and enrollment continue.

0681 PATIENT REPORTED OUTCOME RESULTS FROM A PHASE III RANDOMIZED TRIAL COMPARING NILOTINIB AND IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CML-CP


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Background. Nilotinib has been shown to be a more potent inhibitor of BCR-ABL than imatinib. A randomized Phase III study was conducted comparing these two therapies in adult patients with newly diagnosed Philadelphia Chromosome positive (Ph+) chronic myelogenous leukemia (CML) in chronic phase (CP). Major molecular response by 24 months was significantly improved with nilotinib 300mg BID (71%) and nilotinib 400 mg BID (67%) compared to imatinib 400 mg QD (44%); p < 0.0001 for both comparisons. Adverse event (AE) profiles differed among the treatment arms, and patients treated with nilotinib 300mg BID had the lowest rate of discontinuation due to AE. Aims. To evaluate the patient-reported outcomes, including health-related quality of life (HRQL) and functioning, within the phase III trial. Methods. A total of 846 patients were randomized to receive nilotinib 300mg BID (n=282), nilotinib 400mg BID (n=281), or imatinib 400mg QD (n=283). HRQL was assessed in this open-label study using the Functional Assessment of Cancer Therapy-Leukemia (FACT-Leu) and the Short Form 36 Health Survey (SF-36). The FACT-Leu consists of four general subscales measuring physical, social/family, emotional, and functional well-being and a 17-item leukemia-specific subscale. The FACT-Leu Total score is the sum of all five subscales. The SF-36 assesses eight domains (physical functioning, role-physical, bodily pain, general health, vitality, social functioning, role-emotional, and mental health) that can be combined to create a mental component score (MCS) and a physical component score (PCS). Both questionnaires were administered at baseline, and at 3, 12, and 24 months. Results. Questionnaire completion rates at baseline were 86–92%, and 64–70% at 24 months. High baseline scores reflected that these newly diagnosed patients (median age 47) were in relatively good health and their CML was mostly asymptomatic. FACT-Leu subscale and Total scores were similar across treatment arms at baseline and at follow-up assessments, with differences between treatment arms less than 2 points. At 24 months compared to baseline, FACT-Leu subscale scores were maintained or improved for 57% of patients receiving nilotinib 300mg BID, with 13% experiencing worsening. At each time point

Figure 1. SF-36 scores compared to the US general population.
across treatment arms, differences among the PCS and MCS scores of the SF-36 were less than 1.5 points. Compared to the SF-36 norms for 45-55 year old individuals in the US, mean nilotinib 300mg BID scores were similar to population means for most of the domains at 24 months (Figure). Summary/conclusions. Nilotinib 300mg BID demonstrated superior efficacy including significantly lower rates of progression to AP/BC, lower rates of discontinuation due to AE compared to imatinib, and maintains health-related quality of life and functioning in patients with newly diagnosed CML-CP, at a level nearly comparable to general population normative values. These findings provide a patient perspective and underscore the significant advances in the treatment of Ph+ CML, where patients can achieve deep clinical responses and maintain a near-normal life.

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**0682**

**SOCIO-DEMOGRAPHIC AND CLINICAL DETERMINANTS OF PATIENT-REPORTED SYMPTOM SEVERITY IN CHRONIC MYELOID LEUKEMIA SURVIVORS IN TREATMENT WITH IMATINIB OVER THE LONG RUN**

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Background. The treatment of chronic myeloid leukemia (CML) has changed dramatically over the last decade with the advent of targeted therapies but there is lack of data concerning the long term impact on quality of life and symptoms from the patients’ perspective. Aims. The aim of this study was to investigate the predictive value of socio-demographic and clinical treatment related data of self-reported symptom severity in CML patients undergoing imatinib (IM) over the long run. Methods. A large multicenter case-control survival study including 26 centers was set up to investigate the main research objective and included 422 CML patients who started IM, as first line therapy, in the early chronic phase of the disease. Patients were receiving IM treatment (regardless of dose) for at least three years and were in complete cytogenetic response (CCyR) at the time of study entry. Patient reported symptoms were measured with a previously devised nine items checklist for CML patients undergoing IM treatment. This checklist had a one-week time recall period and all items were rated on a four point likert-type scale (i.e. “not at all”, “a little”, “quite a bit” or “very much”); these measurement characteristics were selected to be consistent with other symptom scales/items included in other psychometric robust measures, widely used in similar long-term studies. The selection of the core symptom domains was based on an extensive literature review, published data on side effects of IM as well as from patients input. Items investigated the following issues: abdominal discomfort, diarrhea, edema, fatigue, headache, muscle cramps, musculoskeletal pain, nausea and skin problems. An overall symptoms index was derived by averaging all symptoms standardized scores. A regression analysis was performed for symptoms index as response variable and a number of potential key covariates were investigated including: gender, age, toxicity, performance status, living arrangements, education, time to achieve a first CCyR, haemoglobin, comorbidity and IM current dose. Results. Fatigue was the most prevalent symptom with 82% of patients reporting it with any level of concern. Nausea and abdominal problems were the less frequently reported symptoms. A univariate analysis identified the following variables predicting symptom severity: gender (P<0.001), age (P=0.002), living arrangements (P=0.007), comorbidity at diagnosis (P<0.001), assuming any other drugs not related to the diagnosis (P=0.006) and having a previous diagnosis of cancer (P=0.036). The final model retained the following variables predicting a lower reported symptom severity: being male (P<0.001), living with a spouse or partner (P=0.027) and not reporting comorbidity at the time of diagnosis (P=0.002). The total variance explained with this model was, however, small being 13% thus suggesting that other unexplored factors might have an important role in determining patient-reported symptom severity. Conclusions. This is the first evidence based data investigating possible clinical determinants of patient-reported symptoms in CML patients suggesting that gender, comorbidity and living arrangements are of importance. Further possible determinants need to be investigated in future analyses.

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**0683**

**A NEW SYMPTOM MEASURE IN CHRONIC MYELOID LEUKEMIA**

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**Background.** Symptom burden is the combined impact of symptoms from disease and treatment on daily functioning. While the severity of symptoms experienced by patients with chronic myeloid leukemia (CML) receiving kinase inhibitor (KI) therapy may be less than with more intense forms of cancer therapy, these symptoms are experienced for years rather than days or months. This may lead to overall equal or greater symptom burden and resultant functional impairment for these patients. A major barrier to effective symptom management in CML is inadequate assessment. In addition, patient-reported outcomes (PROs) can be endpoints in clinical trials to establish treatment benefits. Aims. We aimed to develop a short, easily-understood, valid, and reliable PRO measure of CML symptoms for research and practice. Methods. After providing institutional review board-approved informed consent, 127 patients with CML completed the 13 symptom severity and 6 interference items of the core MD Anderson Symptom Inventory (MDASI) plus 7 CML-specific symptom items (Table 1), generated from patient and expert input, measured on a 0-10 scale (0 = none, 10 = worst imaginable). 85 patients completed the same items 2 weeks later. Patients also answered a single quality-of-life (QOL) question. Demographic and disease information was collected on all patients. Multivariate analysis examined relationships among items. Psychometric procedures determined reliability and validity of the MDASI-CML. Results. Mean subject age was 63.4 years (standard deviation [sd] = 10.57), 54% of the subjects were female, 76% were white, 62% were employed, 99% had an ECOG performance status < 2, and 98% were in chronic phase. 94% were receiving KI therapy (of total receiving KI therapy, 48% were receiving dasatinib, 16% were receiving nilotinib, and 9% were receiving an investigational KI). Mean overall quality of life rating was 8.2 (best = 10, sd = 1.9). Selected symptom severity and interference scores are in Table 1. All items were retained as clinically significant and non-redundant. The reliability index (Cronbach α) and test-retest reliability of the 20 symptom items were 0.94 and 0.95 respectively and of the 6 interference items were 0.94 and 0.91 respectively. The MDASI-CML discriminated between patients who were employed versus those medically disabled and with good versus poor QOL. Symptom severity explained 87% of the variance in interference, with the core symptoms explaining 83% and the CML-specific symptoms explaining 76%. Patients receiving imatinib reported significantly (P<0.048) more severe CML-specific symptoms (mean = 1.93, sd = 1.60) than patients receiving dasatinib or nilotinib (means = 1.27 and 1.03 respectively, sd = 0.99 and 1.30 respectively). Summary/Conclusions. We have validated an analytic tool, the MDASI-CML, for quantifying CML symptoms. The MDASI-CML is being used to assess side effects in treatment trials and to monitor symptoms in clinical care. Additional research on the longitudinal symptom burden, including differences based on type of KI therapy, is needed.
Chronic myeloid leukemia - Clinical 2

0684
LOSS OF MAJOR MOLECULAR RESPONSE (MMR) IS MORE ACCURATE THAN LOSS OF COMPLETE MOLECULAR RESPONSE (CMR) FOR RESTARTING IMATINIB AFTER IMATINIB DISCONTINUATION IN CP-CML PATIENTS WITH LONG LASTING CMR

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3 Hôpital Saint-Louis, Paris, France
4 Hôpital Lapeyronie, Montpellier, France
5 Hôpital Henri Mounier, Créteil, France
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9 Inserm U882, UMR882, U927, U876, Université Victor Segalen, Bordeaux, France

Background. We have reported the results of imatinib discontinuation in 100 CML (Chronic Myelogenous Leukemia) patients in complete molecular response (CMR) for more than 2 years under imatinib therapy (STIM study). The molecular relapse was defined by two consecutive positive values of the BCR-ABL/ABL ratio. Using this criterion, 89% of the patients had to restart imatinib therapy and achieved a second CMR (Mahon et al. Lancet Oncology 2010). However, we identified patients experiencing occasional positive values without a confirmed molecular relapse. We then asked whether the loss of major molecular response (MMR) could be a more accurate criterion for restarting imatinib in an independent cohort of patients. Patients and Methods. Patients were retrospectively analysed. CP-CML patients were eligible if they were in CMR (CMR ≥4.5 log) under imatinib therapy for more than 2 years. Those patients were not enrolled in the STIM study because the study was not initiated or closed or because they experienced one positive value of the BCR-ABL/ABL ratio during the 2 years follow-up. The criterion for restarting imatinib was the loss of MMR. We were then able to calculate molecular relapse free survival using different end-points such as loss of CMR (only one BCR-ABL positivity), loss of CMR using the STIM definition and loss of MMR. Results: 25 CP-CML patients were included in the analysis. Median follow-up is 54.8 months (33-102.7). Sex ratio (M/F) was 53% with a median age of 55.7 years (32-77.6). Sokal score distribution was 39.1%, 34.7% and 26% for low, intermediate and high values respectively. 14 out of 25 patients received interferon therapy prior to imatinib or interferon therapy during the follow-up. Median duration of imatinib therapy was 76.5 months (range 41.2-122.6). Patients were treated for more than 12 months. Their mean OS calculated from the first dose of imatinib was 137 (95% CI 117.6-156.4) months. 18 (29.5%) of them reached CCyR in median time of 16.7 months (95% CI 17.4-47.5). Nine of these patients (14.8%) of patients treated more than one year, 7.6% of the whole cohort) achieved BCR-ABL negativity in nested RT-PCR. CCyR and BCR-ABL negativity in nested reverse-transcriptase polymerase chain reaction (RT-PCR) on overall survival (OS) of patients. Conclusions. Despite numerous adverse effects and treatment failures a significant group of CML patients can gain long-term profit from INF. Unlike the patients with the sole CCyR of whom majority lost CCyR despite continuing INF therapy and later required treatment with TKIs, patients who achieved BCR-ABL negativity in nested RT-PCR had excellent long-term outcome and high probability of operational cure.

Acknowledgement. Study was supported by the grants MSM 6189895223 and MSM 6189895205 of the Ministry of Education Youth and Sports of the Czech Republic.

0686
THE NEW TYROSINE KINASE INHIBITORS FOR FIRST-LINE TREATMENT IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA - SYSTEMATIC REVIEW AND META-ANALYSIS

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Background. Imatinib is considered first line treatment in chronic phase (CP) chronic myelogenous leukemia (CML) patients. Despite the excellent results obtained from the IRIS trial, 30% to 35% of patients discon-
Background. An unfavorable response to imatinib is defined as either failure to achieve a complete cytogenetic response (CCyR) or a major molecular response (MMR) at 12 months or progression to advanced or blast crisis. The aim of this study was to evaluate the incidence and predictors of an unfavorable response to imatinib in a large cohort of patients with chronic phase chronic myeloid leukemia (CML) treated with imatinib as first-line treatment.

Methods. We conducted a retrospective cohort study of 17,312 patients from 200 patients' medical records who were treated with imatinib as first-line treatment for CML at 140 institutions across 15 countries. The primary endpoint was the incidence of an unfavorable response to imatinib. The secondary endpoints were predictors of an unfavorable response. The study was approved by the institutional review board at each participating institution.

Results. The incidence of an unfavorable response at 12 months was 33.9%, with 19.2% of patients achieving CCyR or MMR. The predictors of an unfavorable response included higher baseline BCR-ABL transcripts, higher baseline WBC count, and higher baseline ECOG performance status. Patients with an unfavorable response were more likely to have a lower probability of achieving a CCyR and MMR, and a higher probability of progression to advanced or blast crisis.

Conclusions. An unfavorable response to imatinib is common in patients with chronic phase CML. High BCR-ABL transcripts at diagnosis, higher baseline WBC count, and poor performance status are independent predictors of an unfavorable response. These findings highlight the importance of identifying patients at increased risk of an unfavorable response to imatinib and developing strategies to improve treatment outcomes.

Table 1. Complete cytogenetic response at 12 months.

<table>
<thead>
<tr>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IM</td>
<td>CCyR</td>
<td>0.95</td>
</tr>
<tr>
<td>IM</td>
<td>MMR</td>
<td>0.98</td>
</tr>
<tr>
<td>IM</td>
<td>Progression to AP</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Abbreviations: IM, imatinib; CCyR, complete cytogenetic response; MMR, major molecular response; AP, accelerated phase; BC, blast crisis; OR, odds ratio; CI, confidence interval; p-value, statistically significant.
by comparison IPL in CML patients with stable CCyR and with loss of CCyR. Methods. IPL were detected in 16 CP CML patients with Imatinib treatment duration more than 12 months. The age of patients was 18-78. Male/female ratio was 172/144. Imatinib doses were 400 mg QD (n=207) and 600 mg QD (n=109). Blood samples were collected in 21-27th after the last Imatinib dose intake. All patients gave informed consent before blood sampling. Imatinib concentrations (C trough) were determined by a validated LC/MS/MS method. Results. The obtained results were analyzed in 4 groups CP-CML patients: CML patients in imatinib treatment with 400 mg QD with CCyR and loss of CCyR, with 600 mg QD with CCyR and loss of CCyR (Tab.1). The mean of IPL in CML patients treated with Imatinib 400 mg QD with CCyR was 1162±30 ng/ml vs 651±66 ng/ml in patients with loss of CCyR, with 600 mg QD with CCyR and loss of CCyR (Tab.1). The mean of IPL in CML patients treated with Imatinib 600 mg QD with CCyR was 1709±61 ng/ml vs 878±91 ng/ml in patients with loss of CCyR, with 600 mg QD with CCyR and loss of CCyR (p<0.0001). Conclusions. The IPL in CML patients achieved stable CCyR significantly higher than in patients with loss of CCyR. High level of IPL is important for maintenance of CCyR. Probably the achievement of CCyR in cases with low level of imatinib concentration in plasma is associated with low stability of achieved CCyR.

**Table 1.**

<table>
<thead>
<tr>
<th>Patients</th>
<th>Median Age</th>
<th>Male/Female Ratio</th>
<th>Mean IPL</th>
<th>SD IPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>109</td>
<td>25.8</td>
<td>144/65</td>
<td>2470</td>
<td>619</td>
</tr>
<tr>
<td>207</td>
<td>25.8</td>
<td>172/35</td>
<td>1162</td>
<td>30</td>
</tr>
</tbody>
</table>

The ENESTnd study showed that, vs. imatinib (400 mg QD), nilotinib (300 mg BID) results in superior major molecular and complete cytogenetic responses and time to disease progression as first line (FL) therapy in newly-diagnosed patients with Ph+ CML-CP. Aims. To assess, from a Swedish societal perspective, the direct and indirect costs and quality adjusted life years (QALYs) of FL imatinib vs. nilotinib in newly-diagnosed Ph+ CML-CP. Methods. A literature-based Markov model was developed to estimate the lifetime QALYs and costs of Ph+ CML-CP patients initiating therapy with nilotinib or imatinib. A central model feature is the discontinuation rate from FL therapy, which was based on ENESTnd for the first 12 months and, thereafter, on the rate observed in the IRIS study, stratified by initial 12-month FL responses. Patients discontinuing FL therapy were modeled to receive one additional tyrosine kinase inhibitor (TKI). Diagnosis after FL discontinuation was modeled using published studies. Clinical outcomes and drug exposure were obtained from the ENESTnd study. Non-TKI-drug costs and productivity loss were assumed to increase as disease progressed. Quality of life varied by disease stage and response. Results. Compared to FL imatinib (Table), FL nilotinib results in increases in net discounted FL drug therapy costs, decreases in other direct medical costs and productivity loss, and gains in discounted survival and QALYs. The discounted incremental cost/LY and cost/QALY are estimated at 16 028 € and $18 163 € respectively. In probabilistic sensitivity analysis, 95% of model replications cost $4 450 €/QALY gained. Conclusions. FL nilotinib is cost-effective in Swedish patients with Ph+ CML-CP who are initiating TKI therapy.

**0689**

**COST EFFECTIVENESS OF NILOTINIB VS. IMATINIB AS FIRST-LINE TREATMENT FOR NEWLY DIAGNOSED PATIENTS WITH PH+ CHRONIC MYELOID LEUKEMIA IN THE CHRONIC PHASE (CML CP): SWEDISH PERSPECTIVE**

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Background. The ENESTnd study showed that, vs. imatinib (400 mg QD), nilotinib (300 mg BID) results in superior major molecular and complete cytogenetic responses and time to disease progression as first line (FL) therapy in newly-diagnosed patients with Ph+ CML-CP. Aims. To assess, from a Swedish societal perspective, the direct and indirect costs and quality adjusted life years (QALYs) of FL imatinib vs. nilotinib in newly-diagnosed Ph+ CML-CP. Methods. A literature-based Markov model was developed to estimate the lifetime QALYs and costs of Ph+ CML-CP patients initiating therapy with nilotinib or imatinib. A central model feature is the discontinuation rate from FL therapy, which was based on ENESTnd for the first 12 months and, thereafter, on the rate observed in the IRIS study, stratified by initial 12-month FL responses. Patients discontinuing FL therapy were modeled to receive one additional tyrosine kinase inhibitor (TKI). Diagnosis after FL discontinuation was modeled using published studies. Clinical outcomes and drug exposure were obtained from the ENESTnd study. Non-TKI-drug costs and productivity loss were assumed to increase as disease progressed. Quality of life varied by disease stage and response. Results. Compared to FL imatinib (Table), FL nilotinib results in increases in net discounted FL drug therapy costs, decreases in other direct medical costs and productivity loss, and gains in discounted survival and QALYs. The discounted incremental cost/LY and cost/QALY are estimated at 16 028 € and $18 163 € respectively. In probabilistic sensitivity analysis, 95% of model replications cost $4 450 €/QALY gained. Conclusions. FL nilotinib is cost-effective in Swedish patients with Ph+ CML-CP who are initiating TKI therapy.

**0691**

**ANALYSIS OF BCR-ABL TYROSINE KINASE DOMAIN ABNORMALITIES IN IMATINIB-TREATED CHRONIC PHASE-CHRONIC MYELOID LEUKEMIA PATIENTS BASED ON 2009 EUROPEAN LEUKEMIA NET RECOMMENDATIONS**

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Background. Abnormalities in BCR-ABL kinase domain (KD), such as point mutation, 35 base pair insertion (35INS), and deletion of exon 7 (Del ex7) have been found in chronic myeloid leukemia (CML) patients. Among such abnormalities, point mutations have been known as the clinically most relevant mechanism of imatinib resistance with 40–80% of detection frequency in imatinib resistant patients. 2009 European Leukemia Net (ELN) guideline recommends mutation screening of patients with suboptimal response and treatment failure during imatinib therapy. However, there has not been much information about BCR-ABL KD abnormality status in suboptimal responder. In addition, 35 INS and Del ex7 have not provided their clinical relevance yet al-
though they have been found frequently in CML patients including suboptimal responders. **Aims.** We analyzed abnormalities in BCR-ABL KD from CP-CML patients with suboptimal response or treatment failure to imatinib, and investigated their mutation status and clinical relevance of the abnormalities. **Methods.** This study included 151 CP-CML patients registered in Seoul St. Mary’s hospital since 2002. Serial samples of patients at 3, 6, 12, and 18 months were collected from the Korea leukemia bank in the form of cryopreserved cells or isolated RNAs depending on availability of each sample. Abnormalities in BCR-ABL KD were analyzed using direct sequencing. The 2009 ELN guideline was applied for definition of patient’s response including optimal response, suboptimal response and treatment failure at each time point. **Results.** We analyzed total 302 serial samples (228 optimal, 52 suboptimal and 22 failure samples) from 151 patients at different time points including 3, 6, 12 and 18 months after initiation of imatinib treatment. KD abnormalities were found with difference in each response group. Point mutations were found in 5% of optimal responders, 10% of suboptimal responders and 27% of treatment failures with higher frequency at 6 months in all 3 response groups. Other abnormalities in BCR-ABL KD, including 5INS and Del ex7, were detected in 12% of optimal responders, 17% of suboptimal responders and 5% of treatment failures with higher frequency at 18 months in suboptimal responders and failure group. Interestingly, patients with other abnormalities in BCR-ABL KD showed significantly (p=0.024) higher ratio of turning out suboptimal response; Among 18 optimal patients with 5INS or Del ex7 by 12 months, 72% (n=13) turned out suboptimal responders among 39 optimal responders without any abnormalities in BCR-ABL KD by 12 months. **Conclusions.** Patients with suboptimal response or treatment failure showed tendency of much higher chance of BCR-ABL KD point mutation in comparison with optimal responders, suggesting that mutation screening is important for patients with suboptimal response as well as treatment failure on the basis of 2009 ELN guideline. Especially, optimal responders harboring 5INS or Del ex7 turned out suboptimal response at 18 months than optimal responders without those abnormalities. However, solid clinical relevance of 5INS and Del ex7 requires long-term follow up with large cohort.

**0692**

**PLEURAL EFFUSION IN PATIENTS WITH CHRONIC-PHASE CHRONIC MYELOID LEUKEMIA (CML-CP) WHO RECEIVED FIRST-LINE DASATINIB IN THE DASISION TRIAL: PATIENT CHARACTERISTICS, MANAGEMENT, AND OUTCOMES**

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**Background.** In the randomized phase 3 DASISION trial of dasatinib vs imatinib in newly diagnosed CML-CP, dasatinib continued to show superior efficacy (higher, faster, and deeper response rates) and acceptable safety/tolerability at 18 months of follow-up (Shah et al. Blood 2010; 116: abs 206). Whereas fluid retention was more frequent among imatinib-treated patients, pleural effusion was observed only in dasatinib-treated patients. **Aims.** To perform retrospective and exploratory analyses of patients with drug-related pleural effusion in DASISION, including assessing if pleural effusion impacted efficacy and describing how pleural effusions were managed. **Methods.** In DASISION, after informed consent, patients were randomized 1:1 to receive dasatinib 100 mg once daily (QD; n=259) or imatinib 400 mg QD (n=260). The primary endpoint was confirmed complete cytogenetic response (cCCyR) rate (defined as complete cytogenetic response [CCyR] on two consecutive assessments) by 12 months. Patients with baseline pleural effusion were excluded. Chest x-rays were performed at baseline and at 6 months, or more frequently if indicated clinically. Pleural effusions were graded according to CTCAE v3.0 criteria: grade 1, asymptomatic; grade 2, symptomatic, ≤ 2 therapeutic thoracenteses; grade 3, symptomatic requiring supplemental oxygen, > 2 therapeutic thoracenteses; grade 4, life-threatening, hemodynamic instability. **Results.** After 18 months’ median treatment duration, pleural effusion had occurred in 31/258 dasatinib-treated patients (12%; 3% grade 1, 9% grade 2, <1% grade 3). Median age was higher in patients with pleural effusion (n=51; 59 years; range 28-82) compared with those who did not have pleural effusion (n=227; 44 years; range 19-84). In patients vs without pleural effusion, median dasatinib dose by last follow-up was 93 vs 100 mg/d and Hasford risk score was low in 28% vs 35%, intermediate in 68% vs 45% and high in 10% vs 20%, respectively. In patients experiencing pleural effusion, median time to effusion was 58 weeks, with 78% of effusions occurring more than 8 weeks after start of treatment. Pleural effusions were managed by dose modification (therapy was interrupted in 24 patients and reduced in 13 patients) and/or medical intervention (13 patients received diuretics, 11 received corticosteroids, eight received both diuretics and corticosteroids, and three had therapeutic thoracentesis). Four patients (1.5%) discontinued therapy due to pleural effusion. Within available follow-up, most effusions (84%) have not recurred. Based on consensus from an expert panel, a pleural effusion management algorithm will be presented. Pleural effusion did not seem to impact efficacy: 94% and 65% of patients who had a pleural effusion achieved a CCyR and major molecular response (MMR), compared with 84% and 56% of patients without pleural effusion, respectively. In patients with or without pleural effusion, peripheral lymphocytosis (defined as absolute blood lymphocyte count > 3.6 x 109/L occurring on ≥2 occasions after ≥4 weeks of dasatinib treatment) occurred in 42% vs 19%. **Conclusions.** After 18 months of treatment in patients with CML-CP receiving first-line dasatinib, pleural effusion was predominately mild to moderate in severity, managed by dose modification and/or medical intervention, more commonly associated with lymphocytosis, and did not seem to affect the achievement of CCyR and MMR.

**0693**

**OUTCOME OF PATIENTS WITH CHRONIC PHASE CML TREATED WITH DASATINIB OR NILOTINIB AFTER FAILURE OF SECOND PRIOR TKI**

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**Background.** The TKIs Nilotinib and Dasatinib offer additional therapeutic options for patients with CML who are resistant or intolerant to imatinib. Preliminary results suggest that some patients may respond
to a second TKI used as third line therapy, but little is known about the long term benefit of such an approach. Aim of this collaborative Italian study was to verify the response and the clinical outcome in patients with CML treated with a third TKI after sequential failure of the previous ones. Methods. We evaluated 82 patients with CML, resistant/ intolerant to Imatinib and treated with Dasatinib or Nilotinib, then switched to a third-line TKI after treatment failure; 38 patients were treated with dasatinib and 44 with nilotinib after imatinib failure. Results. A total of 82 patients were treated with sequential TKIs; 62 (75.6%) patients had received interferon-α before starting Imatinib. At the start of nilotinib as second line, 30/84 (36.1%) patients were in CP, 19 in AP, and 26 in BP. 44 (52.4%) patients developed mutations before starting treatment. The lack of durable cytogenetic remission could be explained by the emergence of new kinase domain mutations and a resistant treatment. The use of three sequential TKIs; patients with better response to third TKI were the same patients (28.4%) of patients derived benefit from the use of three sequential TKIs and second generation TKI therapy, while TKD mutations only influenced survival during second generation TKI therapy. The detection rates of Δex7 were significantly dependent on BCR-ABL A→G substitution method (23% by sequencing and 70% by fragment analysis) and on the expression levels of ABL or BCR-ABL. Δex7 was detected more often in samples at diagnosis and resistance compared to samples collected at times of imatinib sensitivity. In addition, Δex7 was detected on ABL not involved in BCR-ABL translocation in 100% of controls and 76% of CML patients. A prospective systematic analysis of the clinical outcome and dynamics of BCR-ABL TKD mutations and ACA during sequential TKI therapies. Our results suggest that the frequency and type of TKD mutation depends on disease phase and TKI applied. For patients with imatinib resistance, mutation and ACA screening may play a role in identifying patients with poorer prognosis. Screening for BCR-ABL TKD mutations is recommended in TKI resistance before changing TKI, because the presence of different mutations may influence the selection of TKI and the therapeutic response. ABL. Δex7 is likely to be unrelated to TKI-resistance since it was abundantly detected in imatinib naive CML patients on BCR-ABL and the alternative splicing is independent from BCR-ABL translocation. In case of sequencing, we recommend using a primer annealing in ABL exon 7, because Δex7 may lead to a sudden deterioration of sequence quality.

0695 Efficacy of Nilotinib versus high-dose imatinib in early chronic myeloid leukemia patients who have unoptimal molecular responses to standard-dose imatinib (re-NICE Multicenter Study)

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Background. Achievement of major molecular response (MMR) is a significant prognostic factor in CML as it has been shown to be associated with longer duration of complete cytogenetic response (CCyR) and long-term progression-free survival. In IRIS study, patients who achieved both CCyR and MMR showed higher progression-free survival rates, compared to those who had CCyR without MMR and those who did not achieve CCyR. Compared to standard dose of imatinib, higher doses of imatinib are expected to yield higher CCyR and MMR rates, and second-generation tyrosine kinase inhibitor, nilotinib also produces high CCyR and MMR rates in patients with CP CML who are resistant to imatinib. Aims. In this study, the efficacy of nilotinib, a high-dose imatinib was investigated in suboptimal molecular response patients who received first line imatinib therapy at a daily dose of 400 mg. Methods. Early CP CML patients who have achieved CCyR but no MMR after at least 18 months and up to 24 months (≤ 18 to ≤ 24 months) on first line imatinib therapy at a daily dose of 400 mg were enrolled in this clinical trial, and informed consents were obtained. Patients were randomized to nitotinib arm (oral dose of 400 mg BID (800 mg/day), and patients received 800 mg/day administered as 400 mg BID in imatinib dose-escalation arm. To assess the drug efficacy, cytogenetics and RQ-PCR analysis were performed at regular intervals, and baseline mutational analysis was conducted for every patient with subsequent mutational analyses performed in patients demonstrating either lack of response or disease progression. Primary endpoint is to evaluate the cumulative MMR rates by 12 months, and secondary endpoints are to evaluate the cumulative CMR rates and time to and duration of MMR and CMR during further 24 month follow-up. Progression-free survival and safety profiles will also be assessed as secondary endpoints. Results. A total of 21 patients were randomized into nilotinib arm (n = 10) or imatinib arm (n = 11). With a median follow-up of 6 months (range, 1 - 24 months), all patients have maintained CCyR without progression to advanced disease, and progressive decrease in BCR-ABL transcript levels was observed in all patients. Cumulative MMR rates by 12 months were significantly higher in nilotinib arm compared to imatinib dose-escalation arm (68.90% vs. 22.10%, P = 0.0274), and patients treated with nilotinib also showed faster molecular response rates, with 5 patients achieving MMR within 3 months of nilotinib therapy. (Figure 1) Although toxicity was observed more frequently in imatinib dose-escalation arm, no patient required dose reduction or discontinuation of therapy due to toxicities in both randomized groups. Conclusions. These preliminary results demonstrate that BCR-ABL transcript levels in suboptimal molecular responders progressively decrease in both

0694 Genetic mechanisms of tyrosine kinase inhibitor resistance in patients with chronic myeloid leukemia


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Background. Additional chromosome abnormalities (ACA), mutations of the BCR-ABL tyrosine kinase domain (TKD) and BCR-ABL splice variants may cause resistance for first- and second-generation tyrosine kinase inhibitors (TKI) in chronic myeloid leukemia (CML) and Philadelphia positive acute lymphoid leukemia (Ph+ ALL). Aims. The aim of the study was to investigate three potential resistance mechanisms during first- and second-generation tyrosine kinase TKI treatment in CML and in Ph+ ALL. Methods. Karyotyping and BCR-ABL TKD mutation screening were performed in 71 imatinib resistant CML and 6 Ph+ ALL patients. Exon 7 deletion (Δex7) was screened by sequencing in all patients at the time of TKI failure and by fragment analysis in selected samples of CML patients and healthy controls. 26 out of 77 patients received second generation TKI. Results. ACA were present in 30/65 (46%) imatinib resistant patients. In 27/77 (35%) imatinib resistant patients, different BCR-ABL TKD mutations were detected. Mutations were found in 25% (12/47) of chronic-phase, 33% (5/15) of accelerated-phase, 71% (5/7) of blast crisis CML and 100% of ALL patients. In second generation TKI resistance, the spectrum of mutations has changed and fewer types of mutations were detected. In nilotinib-resistant patients, 15 different BCR-ABL TKD mutations were detected. In nilotinib-resistant patients, 24 (50%) patients had mutations before starting treatment; 9 patients on dasatinib and 8 on nilotinib had mutations before starting treatment. The best response to the third line treatment with TKI was 13 (15.8%) MMR, 14 (17.1%) CCyR, 11 Pcyr (13.4%), 6 (7.3%) mCyR, 25 (30.5%) CHR and 15 (18.5%) No Response (NR). In the dasatinib group, 14 (41.2%) patients discontinued treatment because of severe toxicity; 10 (27.0%) patients derived benefit from the use of three sequential TKIs; patients with better response to third TKI were the same patients with a better response to the Imatinib and 2TKIs therapy. In this subset of patients, 6 developed mutations that were sensitive to the sequential treatment. The lack of a durable cytogenetic remission could be explained by the emergence of new kinase domain mutations and a change of therapy resulted in an adequate response. Conclusions. Although allogeneic SCT is the treatment of choice in all patients failing 2 TKI, alternative strategies are required for ineligible patients. The use of a third TKI after failure of two previous TKIs induces response in some patients. Longer follow up of a larger series of patients is needed to determine the long term impact of the response.

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nilotinib and imatinib arms at a daily dose of 800 mg with higher and faster molecular responses in nilotinib arm. Through further clinical investigation on a large patient population and longer period of observation, the efficacy of early intervention of suboptimal molecular response using nilotinib or dose escalation of imatinib will be assessed.

0696
OUTCOMES OF CHRONIC MYELOID LEUKEMIA PATIENTS WITH SUBOPTIMAL RESPONSE TO IMATINIB

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Background. Most of the patients with chronic myeloid leukemia (CML) treated with imatinib response favourably. Nevertheless, some patients fail to achieve optimal responses or loose the response. Monitoring endpoints correlate with long-term outcomes, and guidelines have been established to judge response using cytogenetic, polymerase chain reaction (PCR), and mutation testing. The European Leukemia Net (ELN) guidelines classified patients according to dynamic responses in time. Patients with suboptimal response criteria are a heterogeneous group of patients with different outcomes where the best therapeutic options have not been established. Aims. Analyze the evolution of CML patients with suboptimal response criteria (ELN), and identify patients with different outcomes. Patients and Methods. We studied 80 CML patients in chronic phase treated with imatinib with a median follow up of 66 months. The follow up was done following the ELN recommendations. We have classified the patients in two groups according to the moment when suboptimal response was identified. Group 1: Lack of cytogenetic response at 3 months, less than partial cytogenetic response at 6 months or partial cytogenetic response at 12 months; group 2: included patients with complete cytogenetic response who had not achieved major molecular response. Results. Responses to treatment in our patients were: failure 16%, suboptimal response 37% and optimal response 47%. We have identified 30 patients with suboptimal criteria at any time during treatment. Of the 30 patients with suboptimal response, 6 (27%) corresponded to group 1 and 22 (73%) to group 2. The evolution of these patients until last follow up or treatment change was: failure 62% vs 9% (p=0.007), and achievement of late major molecular response 37% vs 54% (p=0.005) for group 1 and 2 respectively. We have found no correlation among failure and classical prognostic factors (Sokal-Index, mutations at the TK domain or imatinib plasma levels). Conclusions. Suboptimal response criteria fail to identify patients with similar outcomes. In our experience, patients with early suboptimal response (group 1) seem to behave as failure, while a high percentage of patients with late suboptimal response (group 2) achieve an optimal response later on.
Background. Imatinib is able to induce sustained responses including complete molecular responses (CMR) in patients with chronic phase chronic myeloid leukemia (CP-CML). Recent results from the STOP IMatinib trial suggest that imatinib may be safely discontinued in patients with long-lasting CMR (Mahon et al. Lancet Oncol. 2010). Actually, 60% of patients had a recurrence of the disease i.e. a molecular relapse which was defined as positivity of BCR-ABL transcripts in quantitave RT-PCR (sensitivity 4.5log), confirmed by a second analysis, in patients who relapsed responded to reintroduction of imatinib. So, we explored the possibility of a second attempt of imatinib discontinuation for the patients who achieved again a prolonged CMR. Patients and Methods. The recommendation was to stop again the treatment using the same dose was from 400 to 600 mg daily. One female had a treatment interruption for 1 and 3 months correspondently for safe breastfeeding. We also report about 4 pregnancy cases in CML CP patients on imatinib dose was from 400 to 600 mg daily, no treatment interruption at conception. There were 19 pregnancy cases on imatinib treatment in 17 females. 15 females were in CP CML, 2 in accelerated phase (AP) at diagnosis. Two of them had subsequent pregnancies: the 1st pregnancy ended with medical abortion at the beginning of imatinib treatment and at the 2nd one was planned during complete molecular response (CMR) and resulted in healthy infants delivery in both cases. Imatinib dose was from 400 to 600 mg daily. One female had a treatment interruption at conception. The general approach was to stop imatinib after pregnancy diagnosis. The females in CMR were monitored by Real-time polymerase chain reaction (PCR) for minimal residual disease (MRD) and did not require any therapy. The supportive treatment was used in case of cytogenetic or hematologic relapse (interferon alpha, hydroxyurea, one woman refused from therapy). One woman received imatinib during the whole pregnancy period. After delivery the women restarted imatinib immediately, 2 women continued the treatment interruption for 1 and 3 months correspondently for safe breastfeeding. We also report about 4 pregnancy cases in CML CP patients on TKI2 therapy who were switched to TKI2 due to hematologic relapse on imatinib. 1 pregnancy in the male's partner on nilotinib ended with premature delivery (the child had severe hyperbilirubinemia). 1 pregnancy (ongoing, 22nd week) in female was diagnosed on imatinib+hydroxyurea therapy, then the patient was switched to nilotinib since 10th week of therapy. 2 pregnancies occurred on dasatinib.

Table 1. Pregnancy outcomes in CML patients on imatinib.

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<th>Year</th>
<th>Pregnancy Outcomes</th>
<th>Gestation Week</th>
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Conclusions. The median time on imatinib prior to the 1st discontinuation was 49 months (range: 32-105) and the median duration of 1st CMR was 31 months (range: 27-61). The 1st molecular relapse occurred with a median of 2.6 months (range: 0.9-8.4) and a second CMR (CMR2) after imatinib re-challenge was obtained after a median of 5.3 months (0-18.9). Results. The median duration of CMR2 was 19 months (range: 3-28). In 10 out of 15 patients (66%) a molecular relapse was observed 2 months in median after imatinib discontinuation (1-14). Among them, 8 were re-treated with a tyrosine kinase inhibitor (imatinib, n=9; dasatinib, n=1). 2 patients with a non confirmed molecular relapse remained free of treatment with a follow up of 12 and 15 months respectively. In 5 patients a prolonged CMR2 after the second episode of imatinib discontinuation was observed with a median follow-up of 24 months (6-49). Conclusions. It is possible to safely attempt to discontinue imatinib for a second time after a sustained CMR2. However, 66% of our patients experienced a second molecular relapse and restarted TKI therapy. At the other hand, 34% of the patients remained free of treatment with either a non confirmed molecular relapse (13%) or a sustained second CMR (20%). A larger number of patients and with a longer follow up will be presented.
DASATINIB EFFICACY AND TOLERANCE IN THE TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) PATIENTS RESISTANT/INTOLERANT TO IMATINIB IN THE CONTEXT OF REAL CLINICAL PRACTICE MANAGEMENT

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Background. Most of the data about dasatinib treatment of CML patients resistant/intolerant to imatinib come from clinical trials. The data from real clinical practice are still scarce. Aims. To evaluate efficacy and tolerance of dasatinib as the second line therapy for CML patients resistant/intolerant to imatinib managed in the context of every day clinical practice. Methods. We analyzed data about CML patients from the defined region stored in a detailed database INFINITY. Non-hematological toxicity was defined as in the START-R,-A,-B,-L trials (Kantarjian, Blood, 2007; CA180-034, CA180-035). We assessed rates and cumulative incidences of complete hematologic responses (CHR), major (MCgR) and deeper responses (CCgR), major molecular responses (MMolR), and a comprehensive set of survivals: overall (OS), progression-free (PFS), progression-free survival (PFS), where also loss of CCgR, failure to second line therapy as provisionally defined by ELN (Baccarani, J Clin Oncol, 2009), and dasatinib discontinuation for toxicity were included, total failure-free (TFS), where also stop of the treatment for any reason was added; and, alternative treatment-free survival (ATFS), reflecting the real proportion of patients remaining on dasatinib despite an event. Among others we evaluated dasatinib toxicity according to CTCAEv. Results. A total of 98 patients (median age at diagnosis 55.5 years, 19-75; at dasatinib start 57 years; 42 males and 55 females) underwent the analysis; 68% were in CP and 32% in AP or BC at the start of dasatinib treatment. Ninety (91.8%) patients were pretreated with imatinib in a median of 24 months (range: 0.2-75). Dasatinib was administered after a median of 39 months (range: 1-175) from the time of CML diagnosis and the median follow-up on dasatinib was 12.9 months (0.2-50.8). Reasons for the second line dasatinib therapy were: resistance (77%), intolerance (14%), and others (9%). Estimated cumulative incidences of CHR, MCgR, CCgR and MMolR at 24 months were 92.1%, 76.2%, 66.7% and 58.5% for patients in CP, and 51.6%, 30.8%, 17.4% and 66.9% for CP patients and 52.2% and 12.9% of CCgR, 59.7% and 19.4% of MMolR. Estimated OS, TFS, PFS, ATFS, and CCS at 24 months were 90.6%, 92.9%, 90.4%, 66.8%, 63.7% and 66.9% for CP patients and 37.2%, 46.8%, 54.2%, 15.1%, 14.6% and 25.1% for AP/BC patients, respectively. Non-hematological and hematological toxicities of all grades occurred in 63.3% and 85.7% of patients, with 26.4% and 48.4% of cases with grade 3/4 toxicity, respectively. In total, 44 patients permanently discontinued dasatinib therapy for various reasons. Twenty one patients died during the follow-up. Conclusions. Dasatinib in second line setting was confirmed as a safe and effective therapy as well as with patients treated generally. The risk of safety management was observed for patients in the centers. As for advanced phases of the disease, the results are still not satisfactory.

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Delaying the initiation of dasatinib after imatinib failure has a negative impact on outcome for patients with CP-CML: results from a European observational study (FORTE, CA180-211)

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Background. After 6-8 years’ follow-up, 45-56% of patients with chronic-phase chronic myeloid leukemia (CP-CML) discontinue first-line imatinib (Hochhaus, Ann Oncol, 2009), primarily due to inadequate response and/or intolerance. Interventional studies suggest that earlier and deeper responses to dasatinib correlate with better outcomes. Also, in resistant patients, time from first detection of imatinib failure to initiation of second-line BCR-ABL inhibitor therapy is a significant predictor of response (Milojkovic et al. Haematologica 2010). However, very limited data have been gathered, to date, from real-life observational studies. Aims. FORTE, a large, real-life, observational, European study, aimed to estimate the relationship between time elapsed from first detection of imatinib failure until initiation of dasatinib and best response to dasatinib, adjusted for other potentially explanatory factors. Methods. Adult patients with CP-CML who had failed imatinib and were treated with dasatinib for ≥2 months were enrolled at 124 sites across 12 European countries. Disease history, response to imatinib, criteria defining imatinib failure and response to dasatinib were collected from patient charts retrospectively and prospectively for up to 6 months. A predefined selection of covariates (gender, age at dasatinib initiation; time from diagnosis to dasatinib initiation; best response to imatinib and last imatinib dose) were entered/removed in a Proportional Odds model to identify factors potentially influencing dasatinib best response over the entire observation period. Results. Of 457 eligible patients, 176 (38.5%) were imatinib intolerant and 52 (7%) imatinib resistant, including 71 patients who were both imatinib resistant and intolerant. Approximately half the patients (51.6%) were male. Median age at dasatinib initiation was 52.7 years; median times from diagnosis to dasatinib and imatinib initiation were 2.2 and 45.1 months, respectively, and Sokal and Hasford scores were intermediate/high in 219/306 (71.6%) and 185/268 (70.1%) patients, respectively. Overall, 51.6% of patients evaluated had achieved at least cytogenetic response (CCyR) or major molecular response (MMR) on prior imatinib. Median time from imatinib failure to dasatinib initiation was 8.8 months and 67.6% of patients received a starting dasatinib dose of 100 mg/day. During the entire observation period, 536/454 patients (74%) achieved CCyR or MMR on dasatinib. An analysis of 443 patients showed a statistically significant effect of time, in months, from imatinib failure to dasatinib initiation on the achievement of a better response to dasatinib (p<0.025), with an estimated odds ratio [95% CI] of 0.987 [0.976-0.998] after adjusting for the effect of time from diagnosis to dasatinib initiation and best imatinib response. The odds ratio suggests that with a 6-month delay in starting dasatinib, there would be a 7.5% decrease in the odds of achieving a better dasatinib response (including CCyR/MMR). The corresponding decrease in the odds with a 12-month delay is 14.4%. Conclusions. Delaying time to dasatinib initiation has a negative impact on response to dasatinib in CP-CML patients with previous imatinib failure. Results from this observational study are consistent with data from interventional trials in resistant patients and underscore the importance of earlier intervention in patients with CP-CML who have failed imatinib.

0701
Clinical bleeding disorders

0702 QUALITY OF LIFE IN CHILDREN WITH HEMOPHILIA A UNDERGOING PROPHYLAXIS OR EPISODIC THERAPY: RESULTS FROM THE ESPRIT STUDY

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Introduction. Bleeding events lead to joint impairments, arthropathy and chronic pain. Prophylaxis was shown to be the most effective method to prevent bleeding events. The ESPRIT study has investigated whether Health-Related Quality of Life (HR-QoL) was improved in patients treated in prophylaxis compared to those on episodic treatment. To compare HR-QoL in children on prophylaxis vs episodic treatment. Methods. ESPRIT study was a multicenter, randomized, comparative, open, trial aimed to evaluate the efficacy of prophylaxis. Forty-five severe hemophilia A patients aged 1-7 years, with no clinical and radiologic signs of joint damage were enrolled and randomized to prophylaxis or to episodic treatment and followed-up for 10 years. Self-reported and proxy-reported HR-QoL was assessed by Haemo-Qol questionnaires at the end of the study. Results. The Haemo-Qol score for both treatment groups was 29.1 (SD=9.3), similar to that reported by parents (mean 28.90; SD=11.2). Patients and parents reported mainly problems in the dimension friends (mean 56.3; SD=22.2), perceived support (mean 52.92; SD=30.2) and dealing (mean 51.15; SD=33.1). A significant difference was found between episodic treatment and prophylaxis for the dimension family (p<0.029), which was more impaired in the episodic treatment group (mean 44.0; SD=22.6) than in prophylaxis group (mean 11.27; SD=8.7). In fact, children on episodic treatment felt often/always more overprotected by their mother (80%) and their father (80%) than those on prophylaxis (11% by their mother; 20% by their father). Eventually 20% of patient on episodic treatments perceived that their parents had to limit their time at work or leisure, 10% felt their parents had to limit their work or leisure sometime, compared to none of the patients on prophylaxis. Discussion. HR-QoL was found to be significantly worse in children on episodic treatment in the dimension family compared to those on prophylaxis. Indeed children complained that parents had to limit their work/leisure time to take care of them: probably prophylaxis gives parents definite reassurance, so that they do not have the need to look after them obsessively.

0703 PROPHYLAXIS TREATMENT IN YOUNG SEVERE HEMOPHILIA A PATIENTS: EFFICACY, FVIII CONSUMPTION, TROUGH FVIII LEVELS AND THERAPY COMPLIANCE

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Background. Long-term prophylaxis is the gold standard treatment for severe hemophilia A patients (pts); it is effective in the prevention of hemophilic arthropathy in children and in young pts. The standard prophylaxis regimen consists of the administration of FVIII ~30 IU kg-1, every other day or three times a week, with the aim of maintaining a level of FVIII >1%. Venous access, especially in children, can be a barrier to prophylaxis; thus, different regimens which involve a lower number of venipunctures are under evaluation. Aims. To evaluate different prophylaxis regimens in young severe hemophilia A pts, and to compare efficacy, FVIII consumption, trough FVIII levels and patient/family’s compliance. Methods. Twenty severe hemophilia A pts (<18 years) started prophylaxis because of either increasing hemorrhages or presence of a target joint. Three prophylaxis regimens were planned: FVIII, 50 IU kg-1 once a week in 2 pts, 50 IU kg-1 twice a week in 12, 50 IU kg-1 thrice a week in 6. The median age of pts at the start of prophylaxis was 6.9 years (1.1-13.5). Results. All pts were treated with rFVIII. Actual rFVIII doses were: once a week, 50 IU kg-1; twice a week, mean dose 46.5 IU kg-1; thrice a week, mean dose 37 IU kg-1. The median annual number of hemorrhages/other bleedings pre-prophylaxis was 4 (1-12) and 5 (1-30), respectively. During the last 12 months of prophylaxis, values recorded: hemorrhages, median 0 (0-2); other bleedings, median 1 (0-2). Mean/median values of trough FVIII levels were 1.1% and 0.7% (0.22%-8%), respectively. Values of trough FVIII >1% were recorded in 5 pts (4 under twice, 1 under thrice a week regimen). No significant differences in concentrate consumption were recorded between twice and thrice a week schedules. There was no difference in the orthopedic score before (median 3; 0-6) and during prophylaxis (median 0.5; 0.2-2). Median follow-up was 12.7 years (2.6-17.3). During prophylaxis, no inhibitor development was recorded; moreover, 4 low-responding inhibitors (titer <5 BU ml-1) which were present before the start of prophylaxis start disappeared. Conclusions. Twice a week prophylaxis can be an alternative regimen to the standard one in young severe hemophilia A pts. Indeed, we found no significant differences both in trough FVIII levels and efficacy between twice/week and thrice/week regimens. Moreover, reduction of venipunctures, especially in small children, improves the compliance of pts and their families.

0704 SONOGRAPHY FOR THE ASSESSMENT OF HAEMOPHILIC ARTHROPATHY OF ANKLES AND Knees - A NEW TOOL IN HAEMOPHILIA CARE

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Introduction. Imaging such as plain film radiography imaging has always been considered an important tool for the evaluation of complications and for therapeutic follow-up of haemophilic arthropathy. Recently, magnetic resonance imaging has been used for its capability of identifying early joint damages. Unfortunately it is expensive, requires time and sedation in small children, limiting its routine and repeated use. Sonography has been increasingly used for evaluation of joint status for its greater feasibility and better sensitivity for synovial changes. Aims. To evaluate the clinical utility of sonography in hemophilic patients. Methods. In order to evaluate the clinical utility of sonography in hemophilic patients, the authors we have retrospectively analyzed medical records of subjects who underwent sonography of ankles and knees at annual check-up from January 1, 2009 to June 30, 2010. The findings were compared to orthopedic evaluation. Results. Overall 325 joints (115 ankles, 210 knees) in 131 patients with haemophilia (aged 6-79 years, median 32 years) were examined: sonography showed abnormalities in 49% of ankles and in 34% of knees. Synovial hypertrophy and cartilage alterations were the most frequently features (96% of abnormal ankles and 86% of abnormal knees, respectively). Prevalence of abnormalities increased with age and defect severity. Only joints of patients on early prophylaxis showed a lower prevalence of changes of patients on delayed prophylaxis or on demand treatment (2/2, 9% vs. 126/303, 42%). Comparison of sonographic and orthopedic findings showed the latter had a lower sensitivity, especially in the evaluation of synovial changes. Aims. To evaluate the clinical utility of sonography in hemophilic patients Methods. In order to evaluate the clinical utility of sonography in hemophilic patients, the authors we have retrospectively analyzed medical records of subjects who underwent sonography of ankles and knees unidentified by the clinical evaluation. For its properties of low running costs and practicability represents a very good tool for a better assessment and monitoring. Therefore, its use should be emphasized in the routine management care of patients with haemophilia.

0705 REDUCED BONE MINERAL DENSITY IN PATIENTS WITH HEMOPHILIA A AND B IN NORTHERN GREECE

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Background. Hemophilia A and B has been associated with increased prevalence of osteopenia or osteoporosis (67-86%) in a few studies. Aims. The aim of this study is to estimate (i) the prevalence of bone disease in hemophiliacs followed-up in the hemophilia centre of Northern Greece and (ii) its association with hemophilic arthropathy, physical activity as well as with the presence of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) infections. Methods. 104 male patients and 44 age-matched controls were screened. Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry.
osteoporosis. Patients <50 years of age, Z-score of <-2 SD was defined as "below the expected range for age", according to the criteria for the definition of osteoporosis in males by the World Health Organization. Results. Ninety-nine patients aged 45.4±15.2 years (85 with hemophilia A, 14 with hemophilia B) were included. Five patients with diseases related to secondary osteoporosis were excluded. Eighteen patients (18%) were suffering from severe hemophilia, 21 (21%) from moderate and 60 (60%) from mild disease. HCV infection was diagnosed in 38 (38%) and HIV in 7 (7%) patients. Low BMD was diagnosed in 26 of 99 patients (26%) being significantly higher than its incidence in controls (20%) (P=0.0001). Seven patients manifested decreased BMD only in LS, 8 only in TH and 11 in both LS and TH. With respect to the severity of haemophilia, decreased BMD was observed in 5 (28%) of those with severe, in 9 (43%) of those with moderate and in 12 (20%) of those with mild disease. With respect to virus infections, 11 (29%) of the HCV and 3 (43%) of the HIV patients manifested decreased BMD. Low BMD was significantly associated with the severity of hemophilia (P=0.0001), the presence of HCV (P=0.01) or HIV (P=0.0001) and physical activity (P=0.0001), in unadjusted analysis. An inverse association between the degree of arthropathy assessed by Pettersson or Arnold-Hilgartner score was evident only for the knees (P=0.032 and P=0.019, respectively). In multiple regression analysis, adjusting for age, BMI, HCV, HIV, number of affected joints and risk factors for osteoporosis, such as smoking, alcohol, previous fractures, family history of osteoporosis or hip fracture, only the degree of physical activity and the severity of arthropathy in ankles assessed by Pettersson score were associated with low BMD. Conclusions. Our study showed a high incidence of decreased BMD in patients with hemophilia, lower than usually reported. The degree of physical activity and the severity or arthropathy are independent risk factors associated with low BMD.

OSTEOPOROSIS IN EGYPTIAN PATIENTS WITH HEMOPHILIC ARTHROPATHY AND ITS CORRELATION WITH SERUM COPPER, MAGNESIUM AND ZINC LEVELS

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Background. Hemophilia is a coagulation disorder characterized by acute and the musculoskeletal system leading to arthropathy and disability. Patients with severe hemophilia are at risk for developing reduced bone density in childhood and adolescence for number of reasons as arthropathy and joint deformities result in prolonged immobilization, reduced physical activity and predispose them for osteoporosis. This can lead to an increasing tendency of bone fractures in patients after trauma. Osteoporosis is a multifactorial disease with particular considerations to calcium, magnesium and other trace elements as copper and zinc. Objective. To find out the presence of osteoporosis in patients with hemophilic arthropathy and its relation to serum levels of trace minerals as zinc, copper and Magnesium. Methodology. Twenty male patients with hemophilia A and twenty healthy age and sex matched controls were enrolled in the study. Evaluation was carried out clinically, functionally and radiologically. The lower limb joint score of ankles and knees was done according to world Federation of Hemophilia. Juvenile arthrits functional assessment report scale was used. Plain x ray on both knees with bone densitometry (DEXA) were done at femoral neck and lumbar spine (L1 - L4) for all patients and controls. Laboratory investigations included Hb, complete liver and kidney function tests, serological screening for HBs Ag and HCV. Serum Calcium, phosphorus and alkaline phosphatase to rule out metabolic bone disorders. Determination of magnesium, copper and zinc levels in serum were done by using the flameless atomic absorption spectrometry. Results. Severity of osteoporosis assessed by DEXA revealed highly significant lower T and Z scores of lumbar spine and neck of femur in hemophilic arthropathy patients versus controls (p < 0.001). T score of neck of femur correlated negatively with vitamin D levels (r = -0.46, p = 0.05), functional assessment score (r = -0.45, p = 0.04) and total xray score (r = 0.46, p = 0.03). There was no significant difference in either T or Z score of lumbar spine and neck of femur between patients with or without hepatitis C virus (p > 0.05). In hemophilic arthropathy patients, a highly significant decrease was found in serum levels of Mg, Cu and Zn compared to controls (p < 0.01) while there was no statistically significant difference as regards serum calcium levels (p > 0.05). Also, serum levels of Cu and Zn correlated positively with Z score of neck of femur (r = 0.61, p = 0.004 and r = 0.83, p = 0.001 respectively). On the other hand, there was no significant correlation between serum levels of either calcium or magnesium and the severity of osteoporosis as measured by T and Z scores (p > 0.05). Conclusion. Osteoporosis represents a frequent concomitant observation in patients with hemophilia which can complicate management of these patients. Screening of young haemophiliacs for reduced bone density is recommended with measuring the serum levels of trace elements as Zn, Cu and Mg for better assessment of the disease.

A REVIEW OF PCC PROPHYLAXIS IN SEVERE FACTOR X DEFICIENCY IN THE REPUBLIC OF IRELAND

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Severe Factor X (FX) deficiency is a rare autosomal recessive bleeding disorder. Patients with FX:C level of <0.01-0.03 iu/ml have a severe bleeding phenotype with haemorrhage with intracranial haemorrhage accounting for 15% of all bleeding events. Prophylactic FX replacement therapy is challenging because there is no purified FX concentrate available. Fresh frozen plasma, solvent detergent plasma and prothrombin complex concentrate (PCC) contain FX but there are concerns regarding possible thrombogenicity. Seven patients aged 7-303 months old with severe FX deficiency are treated with PCC prophylaxis. Six are born to consanguineous parents. One patient was diagnosed by cord blood FX level while five presented with bleeding episodes; 2/5 with gastrointestinal bleeding at day 1 and day 3 of life, 1/5 presented with umbilical stump bleeding at day 4 of life and 1/5 with intracranial haemorrhage at day 1 of life. The seventh patient is from a non-consanguineous relationship and presented with epistaxis at day 3 of life. All seven patients commenced prophylactic FX replacement therapy with PCC in the first week of life. Dosing regimens range from 25 - 60 IU per kilogramme once to twice weekly. 5/7 patients have had central venous access devices to facilitate the administration of PCC. Two patients have had line related thrombosis. 1/7 has Spastic Diplegia secondary to the intracranial bleed at day 1 of life and 2/7 have required extric PCC to treat bleeding episodes secondary to traumatic events. There have been no spontaneous life or function threatening bleeding episodes while on prophylaxis. We conclude that prophylaxis with PCC reduces the number and severity of bleeding episodes in a population with severe FX deficiency.

MANAGEMENT OF MENARCHE AND JUVENILE MENORRHAGIA IN GIRLS WITH VON WILLEBRAND DISEASE AND CONGENITAL SEVERE FACTOR VII DEFICIENCY

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Women with hereditary bleeding disorders are compromised in their lives with many situations increasing demands on hemostasis. Menarche may be the first occasion for the manifestation of the mild bleeding disorder, however, in girls with severe coagulopathy it may lead even to a life-threatening bleeding. Menorrhagia is a well-known complication of childbearing age in patients with von Willebrand disease (vWD), however, in other bleeding disorders this problem is often underestimated. Aim. We report on the management of menarche and juvenile menorrhagia in patients with severe congenital bleeding disorders. Methods. Girls with bleeding disorders and menorrhagia are managed by hematologist and pediatric gynecologist in the comprehensive hemophilia care centres. Pictorial blood assessment chart is used for assessing the intensity of menstrual bleeding. Results. Eight girls with severe congenital bleeding disorders (age 11-13 years) were referred to our haemophilia centre with heavy menstrual bleeding despite factor replacement therapy started in regional hospitals: four girls suffered from severe FVII deficiency (FVII<
bleeding can be successfully achieved with hormonal therapy and an absence of proper management of this situation. The control of menstrual bleeding and the patient family and pediatrician-gynecologist are of paramount importance. Menorrhagia still represents a high-risk situation in these girls. The median duration of the first menorrhagia with Haemate P and Factor VII concentrate or r-FVIIa was 15 days (range 2-25) and 8.5 days (range 2-22), respectively. Subsequent abnormal menstrual cycles despite the use of prophylactic replacement led to the start of hormonal treatment with gestagins in all the girls after a median of the first 3 (2-6) cycles. Prophylactic concentrates and antifibrinolytics during menstruation were used for additional 4-8 cycles, then the stabilization of menses with hormones was achieved. Later on monophasic combined oral contraceptives were used with no further need for replacement therapy. Hormonal therapy was used during the first menstruation and for additional 4-8 cycles, then the stabilization of menses with hormones was achieved. Later on monophasic combined oral contraceptives were used with no further need for replacement therapy. The median of PBAC score is 68; range 56-108 (normal <60), hemoglobin 12-5; range 10.6-13.2 g/dL (normal limit 12.0-13.5 g/dL) and serum ferritin 25; range 10-40 µg/L (normal limit 15-150 µg/L), respectively. Conclusion. The course of the first menstruation is similar in patients with severe forms of vWD and FVII deficiency. Despite availability of high effective factor concentrates the juvenile menorrhagia still represent a high-risk situation in these girls. The preventive approach and close cooperation of hematologists, patient family and pediatrician-gynecologist are of paramount importance for proper management of this situation. The control of menstrual bleeding can be successfully achieved with hormonal therapy and factor replacement can be avoided.

0709 IMMUNE TOLERANCE WITH PLASMA DERIVED FVIII/VWF CONCENTRATE IN BOYS WITH SEVERE HAEMOPHILIA A AND RESISTANT INHIBITORS

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Background. High titre alloreactive FVIII antibodies remain the most challenging complication of severe haemophilia A in countries with access to FVIII concentrate. There is some evidence that using plasma derived FVIII containing von Willebrand factor (pdFVIII/VWF) to induce immune tolerance (IT) increases the likelihood of success. Most data in this field is obtained from patients exposed to a variety of different pdFVIII/VWF concentrates. Aims. We present 5 boys (aged 7-15 years) with high titre inhibitors who have failed at least one attempt at IT with recombinant FVIII (rFVIII) and were then switched to pdFVIII/VWF with a single concentrate (Fanbd, Grifols). Methods. We present a case note review on our cohort as part of a national data collection exercise which is ongoing. The patients and where appropriate the patients’ families gave informed consent. Data collected included, IT regimen, ethnicity, FVIII mutation, Bethesda titres, number of bleeds pre and post switching to Fanbd and concurrent use of any immune suppression. Results. Three of the boys are black African and 2 are Caucasian. They all had historical inhibitor titres of >10BU but at the time of switching to Fanbd 3 of them had inhibitor titres of <5BU. Two boys had had 25-46 months of high dose rFVIII IT prior to switching, 2 boys had failed a previous IT attempt at another centre and were on bypassing agents and the fifth boy had had a relapsed inhibitor. Four boys were given 100iu/kg bd or 200iu/kg od of FVIII and 1 had 80iu/kg 3 times a week. Four of the boys received a total of 6 courses of rituximab (375mg/m2 x 4) and 3 of the boys had mycophenolate mofetil (10mg/kg bd) for 12-15 months (both off-label uses). The mean inhibitor titre pre Fanbd IT was 10.2BU and the mean most recent inhibitor titre was 2.4BU. The mean follow-up is 49 months. Four of the boys continue on daily or alternate daily treatment with Fanbd with measurable FVIII levels at 24 or 48 hours and a gradually decreasing FVIII dose in 3 boys, but only 2 have a inhibitor level of <0.5BU currently (achieved at 51 and 32 months after switching). One boy has stopped IT and is now on daily or alternate daily prophylaxis with an activated prothrombin complex concentrate. None of the patients had any complications from immune suppression. Summary. In this small cohort attempting IT with a single pdFVIII/VWF concentrate we found that all the subjects had a reduction in their inhibitor titre after switching from rFVIII IT and that they had few bleeding problems and were able to attend school and participate in most normal activities. The most rapid reductions in inhibitor titre were observed in the 2 boys who started IT with Fanbd at the same time as having a course of rituximab.
(41/47, 87.2%) in the post-operative period. During the short-term follow-up (10-3 days) or during the long-term clinical surveillance (3 months) no symptomatic or C-US detected thromboembolic events were registered (0/47, 0%, 95 CI 0.7-0.7). In conclusion, in patients with severe coagulopathy undergoing major surgery requiring replacement therapy, the incidence of proven DVT in the immediate or delayed follow-up is low, <1%; in these patients, mechanical antithrombotic prophylaxis seems to be an effective and safe approach.

0712
COMPARISON OF ATTITUDES OF EUROPEAN HAEMOPHILIA CLINICIANS TOWARDS SPORTS ACTIVITIES IN PATIENTS WITH HAEMOPHILIA
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Background. Until the 70s sport activities were not recommended for patients with haemophilia (F VH) due to risks of muscle and joint bleeds. Although sport activities are considered beneficial for physical health, motoric coordination and psychological equilibrium, there are still several obstacles towards sport activities such as worries of the family and limited knowledge of potential benefits. Therefore three surveys into the attitudes of haemophilia specialists towards sports activities in FWHs were conducted in Italy, Germany and the UK. Methods. All haemophilia centres/treaters in Italy (n=49), Germany (n=70) and he UK (n=75) received a questionnaire via email/mail containing the following information: physician’s sociodemographic characteristics, information about the haemophilia centre (number of patients, experience in the field, and cooperation with other disciplines) attitudes towards sports for haemophilic children (recommendation of sports, frequency, sport competition and specific circumstances) and own sports activities. Results. The questionnaire was compiled by 47 centres in Italy (96%), 35 centres in Germany (50%) and 26 centres in the UK (36%). Most of the British haemophilia centres collaborated with a physiotherapist (83%), followed by German (75%) and Italian centres (62%). In Germany one third collaborated with a sports physician, but only one fifth in Italy, while in the UK only one centre did so. In general physicians recommended sports activities for FWH twice a week. Different recommendations were found among countries: a 100% consensus on recommended and not recommend sport activities was achieved only for few sport categories such as swimming, gymnastics and rugby. Conclusions. Attitudes of haemophilia specialists towards sports activities are quite important, since physicians are influential on the decisions of parents. Physicians should support parents to let their children develop normally.

0713
Efficacy and safety of antithrombotic prophylactic therapy with low-molecular weight heparin (LMWH) after orthopedic surgery in hemophilia patients
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Background. Deep venous thrombosis (DVT) is a common post-operative complication in normal subjects undergoing orthopedic surgery of lower limbs. Therefore, thromboprophylaxis with LMWH or other antithrombotic agents is strongly recommended. On the contrary, LMWH thromboprophylaxis after orthopedic surgery is still controversial in hemophilia patients (pts). Aims. To evaluate the efficacy and safety of post-operative anti-thrombotic therapy with LMWH in severe or moderate hemophilia A and B pts, treated with factor concentrate undergoing orthopedic surgery of lower limbs. Methods. Twelve pts [severe/moderate hemophilia A: 9/1; severe/moderate hemophilia B: 1/1; median age 36 years (30-60)], underwent orthopedic surgery because of a significant hemophilic arthropathy. The following surgical interventions were performed: 3 knee replacements, 3 ankle replacements, 3 knee synovectomies, 2 ankle synovectomies, 1 femur fracture reduction. Recombinant FVIII or FIX were administered as bolus infusions at the following dosages: 1) joint replacement: 1 hour before surgery (0h): 100 IU Kg-1; from the 12th to 60th hour (t12h-t60h) after surgery: 50 IU Kg-1 every 12 hrs; from the 3rd to 7th post-operative day: 40 IU Kg-1, 2 bolus day-1; 2) synovectomy: t0: 80 IU Kg-1; t12h-t60h: 40 IU Kg-1, 1 bolus every 12 hrs; from the 3rd to 7th post-operative day: 25 IU Kg-1, 2 bolus day-1; 3) femur fracture reduction: t0: 100 IU Kg-1; from the 12th hour to the 7th post-operative day: 50 IU Kg-1, 1 bolus every 12 hrs, followed by tapering. All patients received post-operatively prophylactic antithrombotic therapy with enoxaparin at a dosage of 50 IU kg-1 day-1, the first administration of LMWH was performed at the 12th hour after surgery and was continued until complete mobilization of the pts (5-7 post-operative days); in case of femur fracture reduction, enoxaparin was given until plaster cast removal, 4 weeks after surgery. Results. Twelve severe/moderate hemophilia A/B patients underwent orthopedic surgery. They were prophylactically treated either with factor concentrates or with LMWH, and no bleeding or thrombotic complications were recorded. Conclusions. Adequate pre- and post-operative replacement therapy, enables the use of LMWH given at lower doses than the standard ones. Hence, thromboprophylaxis with low doses of LMWH is effective and safe in hemophilia pts.

0714
MANAGEMENT OF DENTAL EXTRactions IN EGYPTIAN CHILDREN WITH BLEEDING DISORDERS OPTIMIZING THE USE OF TRANEXAMIC ACID: A SINGLE CENTER STUDY
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Background. Most of the reports in the literature deal with management of dental extractions usually of adults and rarely of children with haemophilia A and B of variable severity whilst few studies discussed pediatric von Willebrand disease (VWD). rare coagulation disorders and platelet disorders.Replacement therapy remains the mainstay of management inspite of its hazards. The use of tranexamic acid, systemically and locally, as a single not adjunctive therapy to reduce the incidence of postoperative bleeding in these disorders is a subject of debate and large studies are not available till now evaluating its use as op- tional or replacement and local measures were proved in Egypt. Aims. To optimize the use of tranexamic acid pre and post dental extraction in Egyptian children with bleeding disorders and so minimizing the use of replacement therapy. Methods. One hundred patients with bleeding disorders who required dental extractions were recruited from the paediatric haematology and dental outpatient clinics, Cairo University Paediatric Hospital over a 3-year period after a consent was obtained. This included 50 children with platelet disorders [45 with idiopathic thrombocytopenic purpura (ITP), 3 children with Bernard Soulier and 2 with Glanzmann’s thrombasthenia]. In patients with ITP, intervention was done with platelet count ≥ 30,000. The study also included 50 children with inherited coagulation disorders [57 children with haemophilia A, 3 children with haemophilia B, 4 with type 1 VWD and one with type 3oWD, one with FV deficiency and one with FV deficieny]. The management protocol was individualized according to the severity of the bleeding disorder and dental history. Patients were divided into two groups according to the treatment regimen group 1(n=83) including patients with mild and some with moderate bleeding tendency receiving only tranexamic acid systemically and then tranexamic acid mouth wash (TAMW) 5% post-extraction and group 2 (n=20) including few with moderate and all severe bleeding disorders receiving replacement therapy to increase the deficient factor to 20% and then TAMW 5% post-extraction. Follow up was done on day one, three and seven post-extraction Results 103 extractions were done in children with a mean age of 11.2 years. Post-extraction complications were reported in 11/103 (10.7%) [6 children with haemophilia A, 1
child with vWD, 3 patients with ITP and 1 patient with thrombocytopenia) who were not required for hospitalization or extra replacement. The patient group on tranexamic acid showed less frequent bleeding complications as compared to those receiving replacement but the latter had a more severe bleeding tendency. Though the protocol was to use TAMW for a week yet bleeding usually stopped after 2 days and all children found the mouthwash easy to use, palatable and very effective. The protocol presented in this study is feasible allowing haemophilic children to be treated on an outpatient basis with containment of cost and relatively low number of haemorrhagic complications especially for mild and some moderate bleeding disorders. Further multicenter controlled studies are recommended on a larger scale especially on children to standardize guidelines for this unique group of patients.

**0715**

**SUCCESSFUL PREGNANCY AND DELIVERY IN PATIENT WITH CONGENITAL COMBINED DEFICIENCY OF FACTORS V AND VIII ASSOCIATED WITH LUPUS ANTICOAGULANT**

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Combined deficiency of coagulation factors V (FV) and VIII (FVIII), known as F5F8D, is an autosomal recessive bleeding disorder characterized by the concomitant reduction in both factors and bleeding symptoms. F5F8D is extremely rare (1:1,000,000) and developed due to mutations in genes lecitin mannose-binding 1 (LMAN1) and multiple coagulation factor deficiency 2 (MCFD2) which encoded proteins involved in the FV and FVIII intracellular transport. Mutation of LMAN1 gene accounts 70% of F5F8D families, whereas mutations in MCFD2 account 30%. In MCFD2 mutations, FV and FVIII are significantly lower than in LMAN1 mutations. F5F8D is characterized by mild bleeding, as the observed FV and FVIII levels are sufficient to prevent major spontaneous bleeding. However, bleeding commonly follow surgery, dental extraction and trauma. Treatment of bleeding requires substitution with FV (FV) and FVIII (desmopressin or FVIII concentrates). Recently, recombinant activated factor VII (rFVIIa) has been used for the treatment of bleeding in F5F8D. The aim of our study is to present clinical, molecular features and treatment of this patient.

**0716**

**GASTROINTESTINAL HAEMORRHAGE IN PATIENTS WITH BLEEDING DISORDER**

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**Introduction.** In recent years improvements in the treatment for individuals with bleeding disorders in the United Kingdom (UK) means the life span of haemophiliacs and people with severe Von Willebrands disease is similar to that of the UK general population. The emergence of a population of healthy adults with inherited bleeding disorders presents a new challenge for their care. They develop medical and surgical diseases not typically seen in this group. In the UK practice is based on guidance from the UK Haemophilia Doctors Organisation and the World Federation of Haemophilia. Registered hospital centres deliver care; however there is ongoing debate regarding the appropriate clinic setting for the treatment of this patient group. The RCH Haemophilia Centre delivers care to 180 registered patients and based on national guidelines has a policy covering attendances in such patients. Aims. To review the presentation, management, investigation and outcome of patients with bleeding disorders that have presented to the RCH with significant gastrointestinal (GI) bleeding between January 2007 and January 2011. Methods. Patient’s details were obtained from the Haemophilia Centre records. Relevant clinical details were extracted from hospital notes. Details of factor concentrate used were retrieved from the laboratory records. Results. There were seven events of GI haemorrhage in six patients with bleeding disorders; 3 moderate haemophiliacs, 1 mild haemophiliac, 1 severe Von Willebrands disease and 1 2A Von Willebrand patient. Duration of bleeding ranged from 1 to 10 days. Three presented with dark blood loss and four with fresh blood loss. Four patients presented out of hours directly to the admissions unit and 2 contacted the haemophilia nurse in working hours. In 6 cases there was documented discussion with on call Haematologist before factor concentrate was administered however in 3 patients there was a delay in administration of factor concentrate. Four patients required blood transfusion. All patients had Factor concentrate; two had continuous infusions and five had bolus therapy. Investigations and management for the GI haemorrhage was in accordance with the RCH guidelines in all but 1. This patient had a barium meal rather than endoscopy. This may have been in part due to a possible risk of vCJD infection and the need to quarantine an endoscope. The diagnoses were varied; 1 haemorrhoids, 2 diverticular disease, 2 colonic polyps, 1 a small bowel bleed and 1 had no diagnosis. All patients were discharged when symptoms settled. Conclusions. Patients with bleeding disorders do present to other specialties with bleeding unrelated to their original diagnosis. These are often emergencies and there is therefore a need for local management. They should be managed according to established protocols for these conditions along with specialist haematology input. It is essential to clearly communicate the practicalities of factor 8 issue, prescribing and administration to ward staff. Some patients presented late with significant bleeding and this may be because they have not needed recent treatment for their bleeding disorders and/or did not think their bleeding disorder was relevant. Continued patient education is important.
costs were expressed in Euro as at 2010. **Results.** Monthly FVIII usage per patient was 8'852 IU (patients on prophylaxis) vs. 3'981 IU (patients on episodic treatment). Assuming an average price per IU of recombinant FVIII concentrates of 0.75€/yearly, the cost for prophylaxis was 79'689€ vs. 35'829€ for patients on episodic treatment. The Incremental Cost-Efficacy Ratio (ICER) for bleeding event avoided in patients on prophylaxis was 7'537€/0'048 IU (p < 0.05). We calculated the ICER for maintaining all joints pristine over the whole treatment period (mean study time: 82 months): it was 201'601.12€/2(23'000.16€/year). **Discussion.** The cost of prophylaxis was more than the double compared to episodic treatment. ICER per bleed avoided showed the need for a high investment of resource (7'537€). On the other hand, the additional cost for maintaining pristine joints in a child with hemophilia was lower than 50'000€/year.

**0718 BLEEDING PROPHYLAXIS WITH AN ANTI-INHIBITOR COAGULANT COMPLEX (AICC) IN PATIENTS WITH HEMOPHILIA A AND INHIBITORS CAN IMPROVE QUALITY OF LIFE: RESULTS OF THE PRO-FEIBA STUDY**

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**Background.** Patients with hemophilia A and inhibitors are at high risk for severe bleeding and progression of joint disease with consequent deterioration of their health-related quality of life (HRQoL). Prophylaxis with bypassing agents has been suggested as a potential therapeutic option in these patients. Method. A prospective, randomized, crossover study (Pro-FEIBA Study) was designed to evaluate safety and efficacy of an anti-inhibitor coagulant complex (AICC) for bleeding prophylaxis in hemophilia A patients >2 years with high-responding inhibitors. The study compared 6 months of AICC prophylactically dosed at 85 U/kg ±15% on 3 nonconsecutive days per week with 6 months of on-demand therapy (target dose: 85 U/kg ±15%). The 2 study periods were separated by a 3-month washout, during which time patients used on-demand therapy. Quality of life in patients >14 years was assessed at the beginning and end of each study period with 2 generic instruments: the Short-Form 36 (SF-36) and the Euro-QoL 5-Dimensions (EQ-5D). **Results.** Twenty-five patients with median age of 30.2 years (range: 16.1-67.9 years) completed at least 1 QoL questionnaire. Sixteen patients completed both the SF-36 and the EQ-5D at the beginning and end of each study period, 1 patient completed only the SF-36, and 1 patient completed only the EQ-5D. A comparison of the 2 study periods showed a trend towards HRQoL improvement favoring prophylactic therapy. The results reached statistical significance (for conventional levels) of the SF-36 physical component summary score. Specifically, the mean difference in the summary scores before and after episodic treatment was -1.7 (SD 7.9) versus -4.4 (SD 8.4) before and after prophylaxis (p=0.025). **Conclusion.** AICC prophylaxis significantly improved HRQoL as compared with on-demand treatment. Larger cohorts and longer follow-up are necessary to confirm these data.

**0719 COST OF IMMUNE TOLERANCE INDUCTION IN HEMOPHILIA A PATIENTS: RESULTS FROM THE ITER STUDY**

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**Introduction.** Despite considerable evidence demonstrating chronic pain, disability and reduced health status in hemophilia patients, little is known about the impact of transitional life events in terms of educational, vocational and relational changes on the disease and its treatment, and patients’ health-related quality of life (HRQoL). For adolescents and young adults with hemophilia independence, self-management of treatment and integration in job life are main concerns. The HyQoLEurope Study aims to evaluate the impact of transitional events on HRQoL. Additionally, key transitional life events like part-time work, living and professional situation will be identified. **Methods.** In this prospective, longitudinal, multicenter, non-interventional study ca. 100 patients aged 14-35 years with moderate or severe hemophilia A using Helixate from 7 countries are enrolled. The study is divided into two phases: a recruitment phase of up to 18 months. In average they report 6 days off at school/work a year. **Results.** Two data-collection phases of 36 months for each individual patient. The following variables are assessed: socio-demographic characteristics, HRQoL (generic: SF-36, EQ-5D; disease-specific: Haemo-QoL, Haem-A-QoL), sports and physical functioning (HEF-Test-O, short EFIC, Norfolk index), living situation, sexuality, spirituality/religious beliefs, treatment adherence and transitional life events. Clinical data on bleeding history, treatment, orthopaedic status, etc. are collected by physicians. All evaluations will be carried out at baseline and yearly over 3 years. **Results.** In Europe 8 regions were identified capturing different socio-economic, cultural and transitional situations (Regions: I: Germany, Austria, Switzerland, II: France, Belgium, III: Italy, Spain). 44 patients are enrolled up to now in Italy (n=12), Austria (n=10), Germany (n=9), France (n=8), Belgium (n=1), Switzerland (n=3), Spain (n=1). Mean age of patients is 24.2 years (SD=6.2), 68% are living with their parents and 63% are working. 81.6% of the patients are severely affected: 28% receive on-demand treatment, 75% on prophylaxis (twice/week). In average they report 6 days off at school/work a year. The HyQoLEurope Study together with a similar project in Canada is the first study investigating the crucial period of transition in the life of young hemophilia patients. Recognition of specific transitional life events and their impact on HRQoL in adolescents and young adults may allow treaters to provide and/or tailor better quality of medical care, improve support of patients’ self management and facilitate a more efficient allocation of resources.
Clinical hemoglobinopathies 2

0721
ASSOCIATION BETWEEN HAEMOLYSIS AND MICROALBUMINURIA IN ADULTS WITH SICKLE CELL ANAEMIA
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Background. Sickle cell nephropathy (SCN) is highly prevalent and potentially life-threatening in sickle cell anaemia (SCA). End stage renal failure is present in around 11% of SCA patients, increasing with age. While significant associations have been found between microalbuminuria and markers of haemolysis in the paediatric population, similar studies in adults with SCA have been inconsistent. Moreover, the role of hydroxyurea (HU) treatment on haemolysis in SCD patients remains unclear. Aims. To investigate the prevalence of microalbuminuria amongst adults with SCA, and to examine their association with haemolysis. Method. This is a retrospective study based on adult patients (> 16 years of age) attending King's College Hospital over a four year period (1st of January 2006 to 31st of December 2009). Steady-state data linked to clinic visits were collected, including haemoglobin (Hb), reticulocyte, bilirubin and lactate dehydrogenase (LDH) levels. Data was also collected on sex, age at clinic visit and alpha-thalassaemia status. Patients were excluded from the study group if they were regularly transfused, or had other causes of albuminuria or chronic renal failure. Analysis was limited to patients with Hb SS and Hb Sβ0 who had at least one ACR value. All laboratory test results were treated as continuous variables and were log transformed if appropriate. Random effects regression was used to allow for multiple observations from each individual. Variables were analysed using both simple correlation and controlling for co-variates (sex, age, genotype-including alpha thalassaemia trait, hydroxyurea use and white blood cell count). Results. The study group consisted of 207 patients (59% female), mean age 31 years (SD +/- 11, range 16-60). Microalbuminuria (ACR >4.5 mg/mmol) was present in 97 (47%) of the group. Using simple correlation, ACR correlated significantly with LDH (p<0.0001 95% CI 0.002-0.003), bilirubin (p<0.0001 95% CI 0.500-1.09), reticulocyte count (p=0.024 95% CI 0.000-0.001), and negatively with Hb (p=0.001 95% CI 0.229 to 0.002). Alpha-globin genotypes were available in 39% patients. Presence of alpha-thalassaemia correlated negatively with ACR (p=0.008 95% CI 0.242-0.124). After controlling for co-variates the correlation with reticulocytes became non-significant, however all other correlations remained significant: LDH p<0.0001 (95% CI 0.002-0.003), bilirubin p<0.0001 (95% CI 0.451-1.05) and Hb p=0.0006 (95% CI -0.246 to -0.061). eGFR (both MDRD and Hoek methods) correlated positively with reticulocyte count using simple analysis (p<0.0001 95% CI 0.027-0.065 and p<0.018 95% CI 0.003-0.028, respectively) and when controlling for co-variates (p<0.0001 95% CI 0.044-0.054 and p<0.0001 95% CI 0.012-0.040, respectively), eGFR correlated negatively with LDH levels (p=0.0001 95% CI 0.000-0.001). Summary. Microalbuminuria is a common complication of SCD and correlates strongly with all markers of haemolysis, the first time that this has been shown in an adult population with SCD. Correlation of bilirubin and LDH with eGFR suggests an association of haemolysis with hyperbilirubinaemia, a known cause of haemolysis in SCA; has a protective effect against microalbuminuria adding further support to the theory that sickle cell nephropathy is linked to the haemolytic sub-phenotype of SCD.

0722
EFFECT OF (6R)-5,6,7,8-TETRAHYDRO-L-BIOPTERIN ON REAL TIME NITRIC OXIDE AND REACTIVE OXYGEN SPECIES GENERATION IN NEUTROPHILS OF SICKLE CELL DISEASE PATIENTS WITH AND WITHOUT HYDROXYUREA THERAPY
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Background. Sickle cell disease (SCD) is associated with decreased bioavailability of nitric oxide (NO) via the reactive oxygen species (ROS)-mediated consumption of this vasoactive molecule. Decreased bioavailability of NO is mainly due to reduced availability of substrate (L-arginine) and cofactor (6R)-5,6,7,8-tetrahydro-L-biopterin (BH4) of enzyme NO synthase (NOS). The NO donor property of hydroxyurea (HU) effectively participates in treatment of SCD. Administration of hydroxyurea and L-arginine increases NO to greater level than either hydroxyurea or L-arginine supplement alone. However, the effect of BH4 on NO/ROS generation in presence of hydroxyurea remains unexplored. Aims. To investigate modulation of real time NO/ROS generation by BH4 in neutrophils of steady state SCD patients and SCD patients on hydroxyurea therapy (SCD-HU), as compared to controls. Methods. Neutrophils from controls, SCD and SCD-HU patients were included in study with informed consent. Real time NO/ROS generation was monitored utilizing specific probes (4,5-diaminofluorescein-2-diacetate (DAF-2-DA;10 µM) for NO, 2,7-dichlorofluorescein (H2DCF-DA;10 µM) for hydrogen peroxide (H2O2) and dihydroethidium (DHE;10 µM) for superoxide anion (O2-)), respectively, using confocal microscopy. To examine effect of BH4 (10 µM), cells were preincubated with BH4 before adding probes and alterations in fluorescence was quantitated by flow cytometry as mean fluorescence intensity (MFI). Preincubation with NOS inhibitor, L-NAME (100 µM), ROS scavenger, superoxide dismutase (SOD;100 U/ml) were also done and their effect on NO/ROS generation was assessed. Results. Flow cytometric studies revealed that NO production was significantly lower in SCD neutrophils as compared to controls (1.5±0.42; 5.3±1.59 MFI, respectively, n=6, p<0.005) but hydroxyurea treatment augmented NO production (3.97±1.17 MFI, n=6, p<0.005) in SCD-HU neutrophils. ROS generation was higher in the neutrophils of SCD patients as compared to neutrophils of SCD-HU patients [5.75±1.71; 3.2±0.72 (H2O2); and 30.1±2.12; 12.5±0.26 (O2-)] MFI, respectively, p<0.005. On pretreatment with BH4, there was significant increase in NO production (6.26±1.18; 5.3±1.59 MFI, respectively, p<0.005) and decreased generation of ROS [56.2±3.5; 40.46±6.11 (H2O2) and 13.66±6.45; 16.4±7.13 (O2-) MFI, respectively, p<0.005) in control neutrophils. NO production increased (1.69±0.08; 1.5±0.42 MFI, respectively, p<0.005) with decreased ROS generation [50.85±4.12; 5.47±6.71 (H2O2) and 15.8±6.11; 30.1±2.12 (O2-) MFI, respectively, p<0.005] in presence of exogenous BH4 in neutrophils of SCD patients. Interestingly, exogenous BH4 did not significantly increase NO levels in SCD-HU patients (3.97±0.17; 4.02±0.32 MFI, respectively) but instead increased ROS generation [79.15±6.21; 62.32±5.85 (H2O2) and 14.58±0.75; 12.53±0.26 (O2-) MFI, respectively, p<0.005]. Alpha-globin genotypes of increased superoxide radicals in neutrophils of SCD-HU patients. Exogenous BH4, instead of augmenting NO further, increases ROS generation and possibly suggests BH4 autoxidation in presence of increased superoxide radicals in neutrophils of SCD-HU patients. 

Table 1. NO/ROS generation in neutrophils of SCD patients.

<table>
<thead>
<tr>
<th>Control</th>
<th>SCD</th>
<th>SCD-HU</th>
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<tr>
<td>MFI</td>
<td></td>
<td></td>
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<tr>
<td>H2O2</td>
<td>3.2±0.72</td>
<td>30.1±2.12</td>
</tr>
<tr>
<td>O2- MFI</td>
<td>12.5±0.26</td>
<td>30.1±2.12</td>
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0723
SICKLE CELL DISEASE DATA BASE: NEONATAL SCREENING OR NOT? A BELGIAN EXPERIENCE

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The objective of this study was to determine the efficiency of the neonatal screening (NS) program for Sickle Cell Disease (SCD) which was introduced in Brussels in 1999 and extended to all maternities in Brussels. It is a systematic screening performed on liquid cord blood. Affected children are referred to a specialized center. We reviewed 146 medical records of patients with SCD born in Belgium and prospectively followed from the time of their diagnosis in three Brussels' Academic Centers and focused on the subgroup of patients older than 3 years of age at December 31, 2007. They were divided into two groups: the NS and the no NS groups. The incidence of major events (first septicemia, first stroke, first severe anemia, number of hospitalization days from 0 to 3 years, and death) was calculated on 50% in the no NS group. The main pathogen remained Streptococcus pneumoniae and there were no resistant strain despite regular prophylaxis. Incidence of stroke was 1.8% (1/55) in the NS cohort compared to 8.2% (4/49) in the no NS cohort. There was no difference in the incidence of the first severe anemia or the number of hospitalization days for both groups. Two deaths occurred in the NS cohort in the very early childhood (septicemia in one and acute severe anemia for the other). These deaths were attributable to no compliance to antibiotic prophylaxis and poor follow-up. Furthermore these deaths occurred in the very early period after NS has been initiated. No death occurred in the NS group since 12 years probably due to better parents' education and comprehensive care. One death was observed in the no NS group (sudden rupture of cerebral aneurysm). In conclusion, NS program is feasible, safe and appropriate to detect SCD. Although the relative small size of our study and the bias due to reported early deaths by infection or severe anemia in the no NS group before the diagnosis of SCD has been done, our results are very encouraging: NS delays the age of the first severe infection and might reduce the incidence of early neurological complications. It also underlines the better outcome with NS program since 12 years probably due to better parents' education and comprehensive care. These data emphasize the need to continue NS for SCD in Brussels and to improve of parental education and comprehensive care. These deaths were attributable to no compliance to antibio-prophylaxis and poor follow-up. Furthermore these deaths occurred in the very early period after NS has been initiated. No death occurred in the NS group since 12 years probably due to better parents' education and comprehensive care. One death was observed in the no NS group (sudden rupture of cerebral aneurysm). In conclusion, NS program is feasible, safe and appropriate to detect SCD. Although the relative small size of our study and the bias due to reported early deaths by infection or severe anemia in the no NS group before the diagnosis of SCD has been done, our results are very encouraging: NS delays the age of the first severe infection and might reduce the incidence of early neurological complications. It also underlines the better outcome with NS program since 12 years probably due to better parents' education and comprehensive care. These data emphasize the need to continue NS for SCD in Brussels and to extend it to all Belgian maternities.

0724
CO-INHERITED α-THALASSEMA SLUMTS THE CLINICAL RESPONSE TO HYDROXYCARBAMIDE IN SICKLE CELL DISEASE

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Background. Hydroxy carbamide (HC), a key therapy in the treatment of sickle cell disease (SCD) reduces transfusion requirement and the frequency of acute pain episodes, mainly through fetal hemoglobin (HbF) induction. Although HbF levels are markedly increased in many sickle patients receiving HC therapy, up to 40% of sickle patients do not respond clinically. Various genetic variants have been implicated, including co-existing α-thalassemia (data not presented in the study). As α-thalassemia is present in approximately 30% of SCD patients, any effects on HC response would have clinical implications. In a separate study we showed that plasma cell-free DNA (cfDNA), a marker of tissue damage, is elevated during sickle acute pain and reduced with HC therapy. Aims. We performed a larger, prospective study to further investigate the effects of co-inherited α-thalassemia on HC response. We wished to expand on our preliminary findings by collecting data on acute hospital admission and combining this with a comparison of cfDNA levels between patients with SCD alone (SCD), co-existing α-thalassemia (α-SCD) and non-SCD patients. Methods: 62 patients were recruited from the specialist hematology clinics at King's College (n=45) and St Thomas' (n=17) hospitals, London. 18 patients had α-thalassemia. All participants gave informed, written consent. 33 patients were already receiving HC therapy, 22 never had HC therapy, 4 commenced HC during the study and 3 were excluded due to intermittent HC therapy. Clinical Data: For patients treated with HC, clinical data (hematological indices, hospital admissions, HC dosing) was collected retrospectively for the year prior to starting HC therapy and prospectively throughout the study from 6 months after commencement of HC therapy. Non HC patients' data was collected prospectively. Blood samples were collected prospectively for cfDNA measurement. Samples were processed using published methods and cfDNA was measured using real-time PCR. Results. We compared data for the SCD and α-SCD groups, on and off HC therapy. The α-SCD group showed significantly higher cfDNA levels, both for patients on HC (p=0.04) and off HC (p=0.04), as compared to the SCD group. There was a smaller increase in MCV (p=0.02) and MCH (p=0.01) in response to HC therapy; smaller reduction in the number of days spent in hospital due to SCD pain (p=0.02). Conclusion. Clinical response to HC (as measured using MCV, MCH and days spent in hospital due to sickle pain) was significantly attenuated in the SCD patients with co-existing α-thalassemia. We also showed that cfDNA levels (a known marker of trauma and organ damage) were higher in the α-SCD group, both on or off HC therapy. This may be related to reduced hemolysis in α-SCD patients. Co-inherited α-thalassemia, present in a third of all SCD patients, should be taken into consideration both in the clinical and laboratory setting when interpreting HC response.

0725
CLINICAL MANIFESTATION OF SICKLE CELL DISEASE IN THE HBS/HBA CHILD: CAN CRITERIA TO IDENTIFY ‘NON HEALTHY’ CARRIERS BE ESTABLISHED?

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Background. Sickle-cell trait (SCT) affects more than 800 million people worldwide (100.000 in Italy) and has been considered as a benign carrier state. Recently, evidence of severe clinical manifestations (renal medullary cancer, exercise related deaths, exertional rhabdomyolysis, venous thromboembolic events) associated with the carrier state have been emerged, particularly in the adult population. Moreover, it has been reported that adults with SCT can develop conditions that are typical of sickle disease (SCD), such as acute chest syndrome (ACS) and vaso-occlusive pain crises (VOC). Less information is available for SCT children, even if an increase in invasive pneumococcal disease and single case observations of VOC have been reported. Aims. To evaluate if clinical features of SCD can develop in SCT children; to determine social, clinical or laboratory predictive factors for precocious development of such complications in SCT children in order to identify a subgroup of SCT carriers that might benefit from close clinical observation or therapeutic options. Methods. All SCT children at the Sickle Cell Clinic of the Pediatric Hematology Oncology Unit receive a full blood test and clinical examination once a year. We performed a retrospective analysis of the charts of SCT children evaluated at our Center from January 2000 to December 2010 to identify admissions or reports of clinical events related to SCD. Social, clinical and laboratory variables were evaluated to identify possible predictors of a more severe phenotype of the SCT child. Informed consent was obtained. Results. 50/163 patients were HbA/HbS, therefore SCT. 23 M and 27 F (86% were African, 10% European and 4% South-American. Mean age at diagnosis was 57 months. 7/50 children had been admitted at least once for some cause of SCD. 26/48 (54%) were admitted for SCD related complications: ACS (1), VOC (2) and haemolytic crisis (1) (Table 1). Moreover, patient n3 (see Table 1) displays frequent VOC that require minor opioid treatment at home. At steady state, children admitted for SCD related complications (Group 1) had higher mean...
Table 1.

<table>
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<tr>
<th>Year</th>
<th>1</th>
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<tr>
<td>Patient</td>
<td>Age</td>
<td>Gender</td>
<td>Outcome</td>
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Blood parameters were recorded at steady state during the second year of life. VOC requiring hospitalizations, ACS and ACS occurring following VOC/ACS were recorded. The latter was ascribed to patients who were not febrile and had a normal chest X-ray at admission for VOC and who subsequently developed ACS with persistence of pulmonary infiltrates. The expression of a hypercoagulable pattern could represent a risk factor for disease severity in children with SCT. Some coagulation tests might be a non-invasive and useful tool to identify a subset of carriers with an increased risk of SCD related complications. These children could deserve a clinical approach similar to that recommended for SCD patients.

Prospective and larger studies are needed to confirm our findings.
REVISITING RISK FACTORS FOR SILENT CEREBRAL INFARCTION IN A COHORT OF LEBANESE PATIENTS WITH SICKLE CELL DISEASE

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Background. Studies from the US and Europe continue to confirm a high rate of silent cerebral infarction (SCI) in children and adults with sickle cell disease (SCD), and highlight several potential risk factors. Data from Middle Eastern countries is scarce. Aims. To prospectively evaluate the prevalence and risk factors for SCI in Lebanese patients with SCD. Methods. This was a prospective study of 135 SCD patients attending two comprehensive SCD centers in Lebanon. Patients with history of overt stroke were excluded. All patients underwent brain magnetic resonance imaging (MRI) as part of their annual surveillance. Complete blood counts, hemoglobin electrophoresis, steady-state serum ferritin, bilirubin, and lactate dehydrogenase levels were determined. Patient charts were reviewed for demographics, transfusion and spleen status. Written informed consent was provided by all patients. Results. A total of 123 patients (87 SS and 36 SThal) were included in the analysis. The mean age was 14.6 ± 10.3 years (range, 2-51 years), with 73 (59.3%) patients being males. Twenty (16.3%) patients had evidence of silent cerebral infarction (SCI) on brain MRI. Patients with SThal had a lower yet statistically similar frequency of SCI as patients SS (11.1 vs. 19.5%, P=0.274). Patients with SCI had significantly older than patients without SCI (mean age 20.2 ± 10.2 vs. 13.5 ± 10.0 years, P=0.007), but had a similar proportion of males (65% vs. 58.3%, P=0.515). A statistically similar proportion of patients were splenectomized (SCI: 10% vs. no SCI: 26.3%, P=0.118) or had persistent splenomegaly (SCI: 40% vs. no SCI: 43.4%, P=0.777) in both groups. Patients with SCI had significantly lower mean HbF% (11.9 ± 5.3% vs. 17.3 ± 11.8%, P=0.019) and higher mean HbS% (79.9 ± 13.3% vs. 69.3 ± 17.4%, P=0.012) than patients without SCI, but had similar mean total hemoglobin levels (9 ± 1.1 mg/dl vs. 9 ± 1.2 mg/dl, P=0.856). There were no statistically significant differences in mean white blood cell counts, reticulocyte count, total or direct bilirubin, or lactate dehydrogenase levels between the two groups. The median number of total life time transfusions and the median steady-state serum ferritin levels were comparable in both groups (SCI: 17 units vs. no SCI: 10 units, P=0.0311) and (SCI: 285.5 mg/ml vs. no SCI: 245.5 mg/ml, P=0.531). On multivariate logistic regression, a 1-% increase in HbS% was associated with a 1.06 increased odds of having a SCI (95% CI 1.01-1.11) and a 1-year increase in age was associated with a 1.06 increased odds of having a SCI (95% CI 1.01-1.10). Conclusions. Silent cerebral infarction in patients with SCD is associated with advancing age and increasing HbS%, but not with anemia, hemolysis, transfusion status or iron overload. SThal patients can have SCI as frequent as patients with SS.

MATERNAL AND FETAL COMPLICATIONS IN SICKLE CELL DISEASE: OUTCOMES FROM A 10 YEAR STUDY AT MOUNT SINAI HOSPITAL IN TORONTO, CANADA

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Background. Sickle cell disease is an inherited recessive hemoglobinopathy affecting nearly 1 in 600 African Americans. The disease is caused either by homozygous substitution of valine for glutamic acid (HbSS), or less commonly compound heterozygous with lysine (HbSC), in the number 6 position of the β globin polypeptide chain causing haemoglobin polymerization and subsequently sickling of the red blood cells in deoxygenated states. This leads to hemolysis-related nitric oxide depletion, anemia and painful vaso-occlusive crises with resultant chronic ischemia-reperfusion injury. While pregnancy was discouraged in the past, recent improvements in patient management, transfusion and clinical care centres in North America, we have undertaken the first known study in Canada to examine these outcomes in our population. Aims. To identify maternal and fetal complications in pregnant women with Sickle Cell Disease, with reference to the Ontario population. Methods. A retrospective study of consecutive deliveries of sickle cell disease patients (SS, SC, HbS/BetaThal) at Mount Sinai Hospital from September 2000 through December 2010 was conducted. Maternal demographics, pregnancy complications including transfusions, mode of delivery, apgar scores and fetal complications were recorded. Patients were identified from the Special Pregnancy Database and the Delivery Databases of Mt Sinai Hospital. Electronic patient records from the Toronto General Hospital, and paper charts from Mt Sinai Hospital were reviewed. Results. A total of 83 pregnant patients: 71 (86%) HbSS, 10 (12%) HbSC and 2 (2%) HbS/BetaThal was reviewed. All had single live births and 32 of 82 patients delivered within 39 weeks. The mean maternal age was 27 years (range 17-38) with a median gestational age of 38 weeks (range 26-42). Twenty two women (27%) delivered before the 37th week and 32 women (39%) delivered by Caesarean Section. Pregnancy complications: preterm premature rupture of membranes 4 (5%), pregnancy induced hypertension 5 (6%), gestational diabetes 5 (6%), hospitalizations for complications in 13 patients (18%) (range 1-4), transfusions 15 (18% of pregnancies) acute chest syndrome 2 (2%), pneumonia 3 (4%), urinary tract infection 4 (3 upper and 1 lower; 5%). The mean birth weight was 2838±734 g. Eighteen neonates (22%) had a birth weight< 2.5 kg. Mean apgar scores at 1 and 5 minutes were 7.9±1.9 and 8.8±0.8 respectively. Fetal complications were few: IUGR 3 (3.6%), oligohydramnios 2(2%), meconium stained amniotic fluid 23%. There were no fetal malformations. Summary/Conclusions. The C-section rate was higher than the 2006/ 2007 Ontario rate for the general population of 26%, but similar to other sickle cell studies reported in the literature with reported rates of 29-36%. Preterm labour and delivery was < 25% in our patients and similar to 17% and 22% respectively, found in the Cooperative Study of Sickle Cell disease but larger than the 2005/ 2006 Ontario rates of 8.6% and 7% respectively. Other pregnancy and fetal complication rates compare favourably to previous reports. Pregnancy in women with Sickle Cell Disease can be managed effectively by an experienced multidisciplinary team including hematologists and high risk pregnancy obstetricians.
ELEVATED TRICUSPID REGURGITANT JET VELOCITY IN LEBANESE PATIENTS WITH SICKLE CELL DISEASE IS ASSOCIATED WITH MORE SEVERE DISEASE, FAMILIARLY CLUSTERED AND NOT RESPONSIVE TO HYDROXYUREA

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Background. Elevated tricuspid regurgitant jet velocity (TRV), a measure of pulmonary hypertension, is associated with increased morbidity and mortality in adult patients with sickle cell disease (SCD). Its significance in a younger population is unclear. Aim. The objective of our study was to find an association between elevated TRV and parameters of disease severity, hemolysis and hydroxyurea (HU). Methods. We conducted a review of all patients with SCD referred to our comprehensive care center. We assessed disease severity by the frequency of vaso-occlusive crises, acute chest syndrome, findings on transcranial doppler screening (TCD), in addition to the presence of retinopathy, stroke, splenic sequestration, proteinuria, and avascular necrosis. Elevated TRV was defined as ≥2.5 m/s. Results. We studied 220 patients with SCD at our institution, 92 (41.8%) patients had a TRV ≥2.5 m/s. Four patients underwent cardiac catheterization, and all of them were found to have an elevated pulmonary artery pressure. The median age was 15 years (range: 3 to 45 years) with a M:F ratio of 1:04. Of the 92 patients, 78 (84.7%) had HbSS, 10 (10.8%) HbSβ-, 3 (3.2%) HbFp, and one has HbSβd. Patients with an elevated TRV were significantly more likely to have at least 3 vaso-occlusive crises per year (42.4%) and recurrent episodes of acute chest syndrome (45.6%) as compared to patients with a TRV<2.5 m/s. Seventeen patients (18.5%) had avascular necrosis of at least one joint, four patients (4.3%) had proteinuria, three (3.3%) had retinopathy, and 2 patients (2.1%) had a stroke. Nineteen patients with elevated TRV underwent transcranial Doppler (TCD) screening and all were within normal limits. Fifty-seven patients (62%) had been on hydroxyurea upon presentation with a mean dose of 18.5mg/kg/day. Currently, sixty-four (69.6%) patients are on HU with a mean dose of 21.5mg/kg/day. Increasing the HU dose by a mean of 3mg/kg/day, did not have any effect on lowering the TRV, but was associated with a decrease in LDH by a mean of 82 IU/L, and a modest mean decrease in total bilirubin (0.5mg/dl), WBC count (2285/cu.mm) and reticulocyte count (1.5%). Hemoglobin and MCV increased by a mean of 0.5g/dl and 8fl respectively. Similar results were seen when using 2.6m/s as a cutpoint. In addition to that, we found that 17 families had 2 or more members with elevated TRV, and patients with a positive family history of high TRV had a higher index of severity for the disease. Conclusions. The prevalence of elevated TRV ≥2.5 m/s was 42% in our population of patients with SCD. While increasing the hydroxyurea doses did result in a decrease in the hemolysis parameters, it did not seem to lower the TRV. Patients with elevated TRV had severe disease as compared to those with normal TRVs. Familial clustering of elevated TRVs was seen in our highly consanguineous population, which might point to an underlying genetic predisposition for developing pulmonary hypertension.

BONE DISEASE IN YOUNG EGYPTIAN PATIENTS WITH BETA-TALASSEMAIA MAJOR AND INTERMEDIA: CORRELATION WITH BIOCHEMICAL, HORMONAL AND GENETIC PROFILES

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Background. Bone disease is an important cause of morbidity in beta-thalassaemia major (TM) and thalassemia intermedia(TI) patients. Thalassemia osteopathy results from a variety of genetic and acquired factors. Objectives: to study the magnitude of bone disease in TM and TI patients and to assess the influence of biochemical, hormonal and genetic factors. Methods. In a cross sectional study, 95 patients (55 TM, 40 TI), aged 13-50 years (mean 17± 3.8 years) were evaluated for BMD by Dual x-ray absorptiometry for lumbar spine and femoral neck. Anthropometric measures and Tanner stage were assessed. The effects of sex, transfusion/chelation program as well as disease duration were evaluated. Laboratory evaluation included: CBC, serum calcium, phosphorus, serum ferritin level, bone alkaline phosphatase, parathyroid hormone and 25-OH-vitamin D. PCR - RFLP technique was used to analyze VDR gene FokI polymorphism. Results. Weight/Height SDS, Height/Age SDS, sitting height SDS were comparable in TM and TI; BMC SDS was lower in TM compared to TI (P=0.02). Hormonal replacement therapy was given to 65% of studied patients. Six TM patients (5%) reported pathological fracture. Bone mineral density (BMD) was reduced in 60% of thalassaemics: 60% had osteoporosis and 30% had osteopenia. In TM patients spine Z-score (-3.3 ± 1.4) was lower than femoral Z-score (-0.68 ± 1.3, p= 0.000). The spine Z score was lower.
in TM compared to TI patients (P<0.05). Negative correlation was found between the age and femur Z-score (P=0.000), femur T-score (P=0.000) and spine Z-score (P=0.000) and spine T-score (P=0.03). Sex was not correlated to BMD. Positive correlation was present between pretransfusion Hb and spine Z score (P<0.05); transfusion frequency and both spine Z score (P<0.000) and Femur T-score (P=0.04). Negative correlation was present between ferritin and spine Z-score (P= 0.015), ferritin and spine T-score (P=0.03), ferritin and femur T-score (P= 0.03), ferritin and femur Z-score (P= 0.04). Positive correlation was found between spine Z score and Height /Age SDS (P< 0.004), sitting Ht SDS (P< 0.03). 75% had low serum calcium, none was symptomatic. 72.5% had low FTH level and 9% had hyperparathyroidism. None had low 25-OH-vitamin D. Bone alkaline phosphatase was elevated in 95% of patients. BMD was not correlated to serum calcium or FTH levels. Spine Z-score was negatively correlated with bone alkaline phosphatase [P<0.001]. The genotypic frequency of VDR FokI gene was: FF 52.5%, Ff 22.5%, ff 25%. BMD femur Z score was highest in FF genotype (P = 0.041).

**Conclusion.** Thalassemia osteopathy is multifactorial but in our community inadequate transfusion and the resulting bone marrow expansion as well as poor chelation resulting in iron toxicity to the osteoblasts are important factors, possibly modified by the VDR gene polymorphism.

**0734 RENAL DYSFUNCTION IN CHILDREN AND YOUNG ADULTS WITH β-THALASSEMIA MAJOR**

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**Background.** Patients suffering from β-thalassemia major have now improved life expectancy due to regular transfusions and chelation therapy, however, chronic complications still develop - some only recently identified. Both tubular and glomerular dysfunction has been reported in a relatively limited number of reports. Renal involvement has been attributed to iron overload, chronic anemia, transfusion rate and chelation therapy, mainly to the newer chelator deferasirox. The exact mechanism of renal injury still remains unclear to a large extent. **Aims.** Evaluation of renal involvement in children and young adults with β-thalassemia major and correlation of findings with iron overload and chelation therapy. **Methods.** Thirty three patients on regular transfusion and chelation therapy, aged 4-20 years (mean age 12±4.7 years), participated in the study. Mean pre-transfusion haemoglobin level was 9±4.07 g/dl. For analysis purposes, patients were divided in 2 groups based on chelation therapy: group A consisting of 18 patients receiving deferasirox and group B of 15 patients receiving other chelation (3/15 patients receiving deferrioxamine, 2/15 patients deferiprone and 10/15 patients combined deferiozinex and deferiprone therapy). The two groups did not differ with regards to age, sex and haemoglobin, but group A presented with lower ferritin values compared to group B (mean 1036±56ng/dl and 1744±592ng/dl, respectively, p<0.001). None of the patients presented additional risk factors for renal involvement. In addition to conventional renal biochemistries, acid-base balance, glomerular filtration based on the Schwartz formula, fractional excretion of sodium (FeNa), tubular phosphorus re-absorption and urine calcium and protein were measured. **Results.** All participating patients had normal blood markers of renal function and electrolyte values, as well as normal acid-base balance parameters. Mean glomerular filtration rate was 125±19.9ml/min/1.73m2, mean tubular phosphorus re-absorption 93±1.9% and mean FeNa 5±4±0.2%. 75.7% of patients (25/33) presented with increased urine calcium with a mean value of 6.4±2mg/kg/24h. 43.8% of patients (15/33) had an increased urine protein level, with a mean of 146±2mg/24h. Protein and calcium urine levels did not differ statistically between the two chelation groups (p=0.85 and p=0.77, respectively). With regards to iron overload, there was a negative correlation between ferritin value and urine protein (r=0.454, p=0.09). Finally, a positive correlation was found between protein and calcium urine levels (r=0.304, p=0.085). **Conclusions.** Study results confirm the presence of renal involvement in thalassemia patients, developing at an early age. In addition, the study demonstrates a high risk of proteinuria in patients with lower ferritin values, possibly due to chelation nephrotoxicity developing at lower iron burdens. Although the study does not demonstrate correlation between tubular or glomerular dysfunction with any of the iron chelation methods, further studies are needed in order to arrive at safer conclusions.

**0735 EVALUATION OF RENAL INVOLVEMENT IN CHILDREN AND ADOLESCENTS WITH SICKLE/BETA THALASSEMIA OF GREEK ORIGIN**


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**Background.** Sickle cell nephropathy encompasses a large spectrum of abnormalities. However, few studies have assessed parameters of renal involvement in patients of young age with sickle cell disease (SCD), while relative literature in sickle/beta-thalassemia (S/b-thal) patients is even more restricted. **Aims.** The aim of the present study was to evaluate the renal function in children and adolescents with sickle/beta-thalassemia of Greek origin. **Patients and Methods.** For the purpose of the study 17 S/b-thal patients, aged 3.5 to 18 years (mean 10.97 ± 4.87 years) were evaluated. Mean haemoglobin (Hb) level was 9 ± 0.7 g/dl. None of the patients was recently hospitalized, was on hydroxyurea or a chronic transfusion program, nor presented additional risk factors for renal disease. In addition to conventional renal biochemistries, estimated glomerular filtration rate, serum cystatin C (Cys C), fractional excretion of sodium (FeNa), tubular phosphorus re-absorption and urine calcium, protein, microalbumin and β2 microglobulin (β2 MG) were measured. Moreover, renal ultrasound was performed. **Results.** Mean eGFR was 142.2 ± 22.5 ml/min/1.73m2, with approximately half of the patients (8/17) presenting with an eGFR of > 150 ml/min/1.73m2. Mean urine specific gravity was 1011.3 ± 3.9. No patient presented with microscopic hematuria or with hypercalcemia. Biochemical urine analysis revealed normal normal excretion and phosphate re-absorption. In all patients serum Cys C and urine β2 MG levels were within normal range. However, 29.4% of patients (5/17) demonstrated impaired glomerular function with proteinuria or microalbuminuria (11.8% and 17.6%, respectively). Regression analysis revealed no correlation between age, annual number of vaso-occlusive crisis, Hb, HMF, eGFR, LDH, Cys C or β2 MG levels with the presence of proteinuria or microalbuminuria. Renal ultrasound was normal in all cases. **Conclusions.** Our study revealed a considerably high rate of proteinuria and microalbuminuria in the young S/b-thal group studied. To the best of our knowledge, this is the first study to specifically assess renal involvement in young patients with sickle/beta-thalassemia and of the same ethnic origin. Of interest is the finding of proteinuria and microalbuminuria in patients during their first decade of life. Given that glomerular damage seems to develop early and irrespective of pain rate in S/b-thal patients, it is recommended that regular testing of relative markers is performed in this patient group.

**0736 PERCUTANEOUS ENDOSCOPIC GASTROSTOMY FEEDS IMPROVE WEIGHT AND BODY MASS INDEX IN CHILDREN WITH SICKLE CELL DISEASE AND FALTERING GROWTH**

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**Background.** Children with Sickle Cell Disease (SCD) frequently experience malnutrition, faltering growth and delayed puberty. Growth failure in children with SCD is linked to increased nutritional requirements, endocrine dysfunction, metabolic derangement and deficiencies in specific nutrients. It is postulated that haemolysis and increased erythropoietic activity lead to increased protein and nutrient turnover and utilisation. Faltering growth is unusual in children with SCD below 2 years old, becomes more apparent by 8-9 years of age, and is seen most frequently in the pubertal years. Nutritional supplementation, hydroxyurea and long term blood transfusions have been shown to improve growth in children with SCD. **Aims.** This study aims assess the impact of instigating Percutaneous Endoscopic Gastrostomy (PEG) feeds on growth parameters in children with SCD and faltering growth. **Methods.** Children attending the Paediatric Haematology clinic at the Royal London Hospital aged 5-16 with SCD and faltering growth (Body Mass Index (BMI) <9th centile, and/or weight static for 6 months), in whom dietary fortification and nutritional supplementation have failed, had a PEG tube inserted. An overnight feed regimen of 500-1000 ml Fruselbin Energy was then instigated over 8-10 hours. Weight and height data was collected at 3 monthly intervals pre- and post- PEG insertion. Weight, height and BMI Z-scores were calculated and change
in Z-scores analysed using SYSTAT 10.2. Results. Gastrostomies were inserted in 12 children between November 2008 and February 2011 (9 boys and 3 girls). The median age at PEG insertion was 14 years (range 11-15 years). There was one death prior to completion of the study unrelated to the PEG. Change in weight, height and BMI Z-scores were calculated and compared pre- and post-PEG insertion. Using the ANOVA Estimate Model there was a statistically significant difference in weight Z-score change (P=0.001, see fig. 1) and BMI Z-score change (P<0.01) after commencing PEG feeds. There was a non-significant positive change in height Z-score (P=0.329). Summary/Conclusion. Faltering growth and maturational delay are common in children with SCD. This study provides evidence that PEG feeding improves weight and BMI Z-scores in children with SCD and faltering growth. Although height Z-scores did not change significantly this may reflect the lag in linear growth versus weight gain, or the small sample size. Pubertal delay was frequently seen and the majority of subjects demonstrated improved pubertal development. Patient and carer surveys demonstrated high a level of patient and carer satisfaction with PEG feeding. We plan to commence a randomized controlled trial of PEG feeding for SCD. This study provides evidence that PEG feeding improves weight and BMI Z-scores in children with SCD and faltering growth.

LONG-TERM EFFICACY AND SAFETY OF DEFERASIROX IRON CHELATION IN 104 PEDIATRIC PATIENTS WITH TRANSFUSION-DEPENDENT ANEMIAS IN TWO UK INSTITUTIONS

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Background. Children with transfusion-dependent anemias require lifelong iron chelation. Deferasirox, a once-daily oral iron chelator, provides an attractive alternative to subcutaneous chelation. Published sub-analyses demonstrate that, compared with adult patients, children achieve slower dose escalations and have differing pharmacokinetics. This may impact efficacy. These concerns, coupled with the requirement for lifelong usage in pediatric patients, provide compelling reasons to monitor the long-term safety and efficacy of deferasirox in children. Aims. To examine the efficacy and safety of deferasirox in 104 pediatric patients with transfusion-dependent anemias in two UK institutions. Methods. Data on 104 patients pediatric patients (<16 years) with transfusion-dependent β-thalassemia and sickle cell disease were retrospectively collected in two UK institutions. Data were collected from initiation of deferasirox therapy (up to 54 months). Efficacy was monitored via serum ferritin (SF) and MRI T2*/R2 data. Iron burden was calculated from annual transfusional requirement. Dosage, toxicity and safety were assessed by examination of contemporaneously recorded patient records and measurement of laboratory parameters, including serum creatinine and liver enzyme levels. Results. The median age of patients was 9y 1m (range 2m -16y). Disease characteristics were: Sickle cell disease (n=45) and thalassemia (n=59). Annual blood transfusion was 188 ml/kg for β-thalassemia and 121 ml/kg for sickle cell disease. Median duration of evaluation for sickle cell patients was 18 months (range 3-54m). Thalassemic patients were followed for a median duration of 24 months (range 12-39m). The mean starting dose of deferasirox for thalassemic patients was 15mg/kg, with a mean dose of 27mg/kg at final follow-up. In sickle cell disease the mean doses were 19mg/kg and 21 mg/kg respectively. Mean (SD) SF at baseline for thalassemic patients was 209 ng/ml (770 ng/ml), 2773 ng/ml (967 ng/ml) for sickle cell disease. Mean SF was significantly reduced over time (p=0.005). The change in SF over time was inversely correlated with the dosage of deferasirox for the thalassemia cohort (Fig. 1).
ROLE OF ETO2 IN THE EPIGENETIC REGULATION OF ERYTHROID GENES

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Background. Developmental control mechanisms often utilize multimeric complexes containing transcription factors, coregulators, and additional non-DNA binding components. It is challenging to ascertain how such components contribute to complex function at endogenous loci. We recently analyzed the function of components of a complex containing master regulators of hematopoiesis (GATA-1 and Scl/TAL1) and the non-DNA binding components ETO2, the LIM domain protein LMO2, and the chromatin looping factor LDB1. We revealed that ETO2 and LMO2 regulate distinct target gene ensembles in erythroid cells. Furthermore, it was found that ETO2 commonly represses GATA-1 function via suppressing histone H3 acetylation, and also regulates methylation of histone H3 at lysine 27 (H3-trimK27) at select loci, which suggested that ETO2 might be an important determinant of the erythroblast epigenome (Fujiwara et al. PNAS. 2010). Aims. We investigated the role of ETO2 in the epigenetic regulation of erythroid genes. Methods. CBFA2T3 miRNA (which encodes ETO2 protein) was cloned into pcDNAs.1 (Clontech) and Flexi HaloTag vector (Promega), and ETO2 was transiently overexpressed in K562 cells using Amaza nucleofection technology® (Amaza Inc.). Quantitative ChIP analysis was performed using anti-acetylated H3K9 (abcam), anti-trimethyl H3 (lys 9 and 27) (Millipore) and anti-Myc (Santa Cruz). To induce erythroid differentiation of K562 cells, hemin was treated at a concentration of 50 μM for 24h. For transcription profiling, human whole expression array was used (Agilent). Gene Ontology analysis was based on DAVID software (http://david.abcc.ncifcrf.gov/home.jsp). Results. Overexpression of ETO2 in hemin-treated K562 cells resulted in decreased ratio of benzidine-staining positive cells, suggesting ETO2 reverb the erythroid differentiation of K562 cells. Next, we conducted microarray analysis to characterize ETO2 target gene ensemble in K562 cells. The analysis demonstrated that 599 genes were downregulated in the ETO2-overexpressed cells (>2 fold). To test what percentages of ETO2-repressed genes could be direct target genes of GATA-1 or GATA-2 in K562 cells, we merged the microarray results with ChIP-seqprofile for GATA-1 and GATA-2 peaks, respectively (Fujiwara et al. Mol Cell, 2009), and demonstrated 25.1% and 40.5% of ETO2-repressed genes contained significant GATA-1 and GATA-2 peaks in their loci, respectively. Gene Ontology analysis among ETO2-repressed genes revealed significant enrichment of genes related to “oxygen transport/ hemoglobin complex” (p=0.00128). Overexpression of ETO2 in K562 cells resulted in the significant decrease in the expression of globin genes such as HBG, HBB, HBde, HBA, HBM and HBz by quantitative RT-PCR. Quantitative ChIP analysis revealed ETO2 occupancy at globin HS2. Furthermore, the overexpression significantly increased H3-trimK27 and reduced acetylated H3K9 at γ-globin promoter. Co-immunoprecipitation analysis revealed the interaction between ETO2 and EZH2, a member of polycomb repressor complex responsible for H3-trimK27-mediated transcriptional repression. We are currently analyzing the mechanism of ETO2-dependent transcriptional repression and how ETO2-dependent histone marks are established in erythroid cells. Conclusion. In conjunction with the evidence that ETO2 binds histone deacetylases and associates with GATA-Scl/TAL1 complex that binds epigenetic modifiers, ETO2 appears to have important roles in establishing the erythroblast epigenome. We consider this is important from the perspective of elucidating mechanisms of hematopoiesis and leukemogenesis.

ANTIBODY ARRAY-BASED PROTEOMIC PROFILING OF CHILDHOOD ACUTE LEUKEMIA

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Background. Mapping of signaling pathways and searching for new prognostic molecular markers is a promising approach to design specific treatment of leukemia while minimizing the adverse effects. Large-scale proteomic profiling is expected to yield more direct answers to functional and pathological questions than does transcription analysis of mRNA. Performing large-scale profiling on primary leukemia samples and understanding the effect and functional consequences of targeted anti-cancer agents requires high-throughput technologies as well as novel analytical strategies. Here, we employ bead-based antibody arrays, which can follow changes in protein expression levels that may associate with clinical presentation and therapy efficacy of acute leukemias. Methods. Cellular proteins are separated by a differential detergent treatment to cytoplasmic, membrane and nuclear proteins, labeled with biotin and subjected to size-exclusion chromatography to obtain 24 fractions. Fractions are incubated with a mixture of 1700 color-coded beads each carrying antibodies against all possible protein target. Mixture of all beads binding their specific proteins is analyzed by flow cytometer that resolves beads’ color-code and records the amount of captured protein. This novel concept has several advantages: 1) High level of multiplexing by color-coded beads (1700 bead types) 2) Additional information based on protein size profiles (e.g. protein-protein interactions) 3) Flexibility in array composition (custom selected e.g. phospho-specific antibodies) 4) Semi-automated analysis (gating, quality control, normalization) Algorithms for semi-automated analysis are implemented in R-project environment. It allows for color-code interpretation, captured protein signal read-out and downstream batch analysis including protein size recognition and clustering of results. Array performance was tested on ten representative B-cell precursor (BCP-ALL), T-cell acute lymphoblastic leukemia (T-ALL) and acute myeloid leukemia (AML) cell lines. Next, 80 bone marrow specimens of childhood leukemia at diagnosis were analyzed.

INTEGRATION OF GLOBAL SNP-BASED MAPPING AND EXPRESSION ARRAYS WITH MICRONA PATTERNS REVEALS Deregulation OF MIR-370 AND PERMITS THE IDENTIFICATION OF ITS TARGET GENES IN ACUTE MYELOID LEUKEMIA

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Deregulated microRNA (miRNA) expression has been largely reported to play a crucial role in tumorigenesis. Recent studies have shown different mechanisms leading to miRNA deregulation in cancer: mutations, chromosomal translocations, epigenetic alterations, and defective miRNA biogenesis; however, alterations affecting miRNAs by DNA copy number variations (CNV) remain poorly studied. Our aim was to identify if CNVs affect the expression levels of miRNAs in acute myeloid leukemia (AML), modulating the expression levels of their target genes. We analyzed in a matched 16 myeloid cell lines by an integrative approach including high resolution SNPs arrays, miRNA expression arrays, and quantification of 250 miRNAs by real-time PCR. We found correlation between the expression levels of 19 miRNAs and CNVs affecting the genomic regions in which these miRNAs are located: 16 miRNAs were upregulated and located in two genomic regions of amplification (11q24 and 14q32), and 3 miRNAs were downregulated and located in regions with genomic depletions (13q14 and 9p21). These results prompted us to analyze the expression levels of their predicted target genes. After performing a whole genome expression analysis in the cell lines, we obtained a set of candidate genes whose altered expression (B<0) may result from deregulation of 9 of these 19 miRNAs. The differential expression of these selected 9 miRNAs and their predicted targets were validated by QRT-PCR. Three miRNAs had NF1 as a potential target gene; therefore, we analyzed whether miR-570, miR-637, or miR-494, all located on 14q32.31, could regulate NF1. The AML cell line HL-60, with low expression of the miRNAs was chosen as cell model for miRNA overexpression experiments. By QRT-PCR confirmed overexpression of these microRNAs after transfection with the corresponding pre-miRNAs. Western blot analysis showed that NF1 levels decreased after miR-570 overexpression. No changes in NF1 levels were observed after ectopic expression of miR-579 and miR-494. Furthermore, transfection with pre-miR-NF1(3’UTR) in cells ectopically expressing miR-370 showed decreased luciferase reporter activity, indicating that miR-370 binds to 3’UTR of NF1 regulating its expression. These results highlight that presence of copy number alterations affecting miRNAs represent an alternative mechanism to deregulate the expression of genes with importance in myeloid leukemia development. Further studies are in progress to identify other genes with importance in AML and deregulated by CNVs affecting miRNA expression. e-mail address: laura_garcia_ortiz@hotmail.com
Results. Antibody arrays can accurately determine lineage assignment of leukemia cells (BCP-ALL, T-ALL, AML) in cell lines as well as in patient samples. In parallel to surface proteins, intracellular proteins are quantified (transcription factor Ikaros, E2F, Pax-5; cell cycle machinery). Patient samples. In parallel to surface proteins, intracellular proteins are quantified (transcription factor Ikaros, E2F, Pax-5; cell cycle machinery).

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Reference

0741 MICRO-ARRAY AND 2D-PAGE ANALYSIS REVEAL DIFFERENTIAL REGULATION PATTERNS OF ANTI-CD20 ANTIBODIES GA101 AND RITUXIMAB IN MCL

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Mantle cell lymphoma (MCL), an aggressive B-cell NHL is characterized by a poor long-term prognosis with a median survival of 3-5 years. Obinutuzumab (GA101), a Fab-engineered type II CD20 humanized IgG1 antibody has been shown to result in higher direct cell death induction, increased ADCC and a significant higher cytotoxicity when compared to type I anti-CD20 antibody Rituximab. We conducted an in vitro study to investigate possible differences in downstream signal pathways of the antibodies. Using sensitive MCL cell lines Rec-1 and Granta-519 we evaluated the effect of GA101, rituximab and the combination of both antibodies on cell viability and proliferation. Granta-519 and Rec-1 were treated at a cell density of 5x10^5 cells/ml with GA101 or rituximab at a dose of 10 μg/ml. Samples of 5x10^6 cells were harvested and processed for 2D-PAGE analysis after a 4h exposure. Distinct protein spots with altered expression after antibody treatment from untreated controls were identified and analyzed by mass spectrometry. The antibody micro-array study revealed 40 unique differentially expressed protein spots after GA101, rituximab or combination of both antibodies. Ingenuity Pathway Analysis of the identified genes was performed to elucidate downstream pathways. Mono-treatment with GA101 achieved 70% cell reduction in Granta-519 and 40% in Rec-1. In contrast, rituximab led to 25% and 5% cell reduction in Granta-519 and Rec-1. Combination treatment of both antibodies led to a cytotoxicity comparable to rituximab mono-exposure. Analysis of 2D-PAGE protein maps revealed 40 and 39 distinct differently expressed protein spots after GA101 and rituximab treatment. Analysis revealed that 25 of these protein spots were commonly altered after both antibodies (CCDC158, MACF1, RAB39, RAD23B). 17 (ENO1, MKI67, NPM1, HSPA5) and 16 proteins were uniquely altered after GA101 or rituximab treatment only (e.g. DST, G3BP2, LMO7, PSMD13). Micro-array analysis showed 2-3 (Granta 519) to 14-78 (HBL) modulated genes after antibody exposure in all five MCL cell lines. Distinct sets of candidate genes after rituximab (BCL2A1, CHL1, LILRA4, LPL, LY9, RHEBL1, SROX1, WNT3) and GA101 (EGR2, EGR3, NPT1C1, SPRY2, ZBTB24) were affected in multiple cell lines. Proteome and transcriptome-based analysis depicted different sets of candidate molecules, which were mapped to common cellular functions including e.g. "cell death", "cellular growth and proliferation" and "cell cycle". Interestingly, combination of GA101 and rituximab resulted in a rituximab-like expression pattern, both on RNA and protein level. Proteome and transcriptome-based experiments showed antibody-specific downstream expression patterns of GA101 and rituximab. These results might represent the molecular basis of the superior effect of GA101 in comparison to rituximab. Combined antibody treatment with GS-1599 also resulted in a rituximab-like expression pattern that confirmed our previous experiments on cell viability and proliferation. Proteomic screening identified a group of proteins relating cellular stress response mechanisms and cellular energy metabolism to the treatment of the therapeutic antibody GA101. These results indicate a possibly involved autophagy mechanism after GA101-treatment and a stress response mechanism effecting regulation of cellular metabolism. These results will provide a further step into identifying molecular-based rationales for new combined therapeutic approaches to treat MCL.

0742 ULTRA DEEP AMPLICON SEQUENCING OF TET2 IN PEDIATRIC MDS/JMML SPECIMENS DETECTS SEQUENCE VARIANTS AND A NOVEL MISSENSE MUTATION

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Background. MDS is a clonal bone marrow disorder characterized by cytopenia due to ineffective hematopoiesis and a high risk of evolution to AML. MDS is mainly a disease of the elderly and is rare in pediatric patients. The WHO classifies MDS in several subgroups in the first place based on morphologic assessment of bone marrow cells. Even if similar morphological diagnosis is used to identify MDS it is understood that pediatric MDS are quite different from adult MDS. Since some years genomic aberrations in MDS are reported and aid in improving molecular-risk classification. Mutations in the TET2 gene are described in MDS and MPN with percentages varying from 12-22% and in AML TET2 mutations found in 23% of patients have prognostic impact and are significantly associated with older age. In fact, several studies have reported that TET2 mutations were not identified in pediatric MDS specimens. NGS (NextGenerationSequencing) 454 amplicon sequencing offers a fast, reliable and highly sensitive method of screening for sequence variants and is currently investigated for reproducibility across laboratories. Aims. To set up a platform for NGS sequencing for 454 amplicon sequencing of pediatric leukemia and MDS/JMML specimens and to verify in the first place the occurrence of TET2 mutations in pediatric MDS/JMML.

Methods. In the context of the InterLaboratory Robustness of Next-Generation Sequencing study (IRON) nine pediatric patients, 6 with diagnosis of JMML and 3 with MDS were sequenced for 27 amplicons covering all coding exons of TET2. Primers pairs included a 10-base molecular identifier barcode sequence (MID) to recognize each specific patient. We used Genome Sequencer Junior instruments (Roche Applied Science). All data was generated using the GS Junior Sequencer Instruments Software and sequence alignment and variant detection was performed using the GS Amplicon Variant Analyzer (AVA) software version 2.5 (Roche Applied Science). Sequencing analysis by Sanger sequencing was used to confirm mutation detection in 454 ultra deep screening. Results. We generated amplicon ultra deep sequencing of 27 amplicons of TET2 gene for 9 MDS/JMML patients with a median coverage per amplicon of 650-fold. In total we identified 68 variants in TET2 gene occurring in more than 1% of sequence reads. Of these variants, 6 were known SNPs in MDS and JMML patients with a coverage of > 40% (40-100%) of reads per patient (rs161749960, rs12498609, rs17255672, rs2454206 and rs34402524). In one MDS patient, a 16 years old boy we found a novel missense mutation C/T in exon 11 of TET2 at aminoacid T1980I (ENST 00000380013) (RMA = 43.91% of reads). The mutation was confirmed by Sanger sequencing. Summary/Conclusions. Ultra-deep amplicon sequencing offers a fast and reliable approach for mutation detection and can be basically implemented in mutation screening of candidate genes in leukemia diagnostics laboratories. We have counter currently detected a novel TET2 mutation in an adolescent boy with MDS seemingly indicating that TET2 mutations are rare in pediatric MDS/JMML but may be found in the upper age group of pediatric patients.

0743 CD209 GENE POLYMORPHISMS FOR PREDICTION OF SUSCEPTIBILITY TO INVASIVE PULMONARY ASPERGILLOSIS INFECTION

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Invasive Pulmonary Aspergillosis (IPA) is the most common cause of infection-related deaths in patients with haematological cancers. Despite
the availability of new antifungal drugs, the incidence of IPA is rising as a result of a wider use of broad-spectrum antibiotics, novel immunomodulatory therapies and an increasing proportion of susceptible patients such as solid organ transplantation recipients or critical care patients. 

Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin (DC-SIGN), Dectin-1 and Dectin-2 are transmembrane C-type lectins that are involved in the recognition of fungal pathogens. The recognition of Aspergillus conidia by dendritic cells or macrophages as well as the chemotactic activity mediated by CCL2/CCR2 ligand-receptor axis, are critical steps in the defense response against Aspergillus. It has been suggested that both processes might be, at least in part, genetically determined. Thus, the aim of this study was to assess whether the presence of single nucleotide polymorphisms (SNPs) within DC-SIGN, Dectin-1, Dectin-2, CCL2 and CCR2 genes influence on the risk of Invasive Pulmonary Aspergillosis. A tag SNP approach resulted in a selection of twenty-seven tagging polymorphisms that were genotyped in 217 high-risk patients with haematological malignancies. Out of 217 haematological patients, seventy patients were diagnosed with proven or probable IPA (following the EORTC/MSG criteria, update 2008) and the remaining 147 patients were classified as not infected. Our results revealed that patients bearing the DC-SIGN_rs4804800_G, DC-SIGN_rs11465834_T and DC-SIGN_rs7248637_A alleles had an increased risk of IPA infection (per-allele OR=2.73, 95%CI 1.53-4.5; PTrend=0.0005 and OR=2.08 95%CI 1.16-3.72; PTrend=0.013, respectively). In addition, carriers of the DC-SIGN_rs7252229_C showed a slightly increased risk of IPA infection (per-allele OR=1.68 95%CI 0.94-3.0; PTrend=0.078). Likewise, patients harbouring the DC-SIGN_rs4804800_G or DC-SIGN_rs11465834_T alleles showed an increased frequency of galactomannan positivity that those carrying the A or G allele, respectively (p=0.06 and p=0.08). These results strongly suggest that DC-SIGN polymorphisms might modulate the risk of IPA infection and might be useful as biomarkers for patient stratification and to develop personalized treatment strategies. Nonetheless, these results need to be confirmed in larger cohorts of haematological patients.

0744
LEUKEMIA GENE ATLAS - A PUBLIC PLATFORM TO SUPPORT RESEARCH AND ANALYSIS OF MOLECULAR DATA OF LEUKEMIASE. K Hebestreit, S Gröttrup, HU Klein, C Ruckert, C Bartenhagen, M DuGas
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Background. The number of published studies regarding leukemias has been increasing enormously over the last few years. A vast amount of molecular data is collected, analyzed and published every year. Nevertheless, meta-analyses and comparisons of published studies are performed rarely. For researchers it is time consuming to get an overview of the range of published data and results. To obtain an insight into published data or to re-analyze data, e.g. to compare it with ones own data and results, is demanding for researchers who may lack the statistical analysis knowledge. But the expression of these molecules in different types of leukemia is largely unknown. By using an R-based approach we have systematically analyzed the expression of the three IGF2BP coding genes in normal hematopoietic tissues and diverse hematopoietic malignancies. We show that low IGF2BP1 and IGF2BP3, and high IGF2BP2 expression are characteristic to donor bone marrow (BM) and peripheral blood (PB). Myeloid malignancies - acute myeloid leukemia (AML) and chronic myeloproliferative neoplasms (MPN) - essentially retain the “normal” IGF2BP expression profile. In contrast, lymphoid lineage neoplasms - acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM) - are associated with characteristic perturbations of IGF2BP expression pattern (Figure 1a). Namely, all lymphoid malignancies tend to upregulate IGF2BP1 when compared to the normal corresponding tissue and myeloid lineage malignancies though it was statistically highly significant only in the case of CLL and MM (p<0.001) as revealed by 1-way ANOVA. In addition, CLL also shows a remarkable variation in IGF2BP3 expression levels while MM appears to be virtually negative for this transcript (Figure 1a). The most prominent perturbations were identified in ALL, where IGF2BP1 and IGF2BP3 varied over five and four orders of magnitude, respectively. As ALL is comprised of biologically and clinically different disease entities IGF2BP profiles were further reanalyzed with respect to this (Figure 1b). We have identified significant associations of overexpressed IGF2BP1 with ETV6/RUNX1-positive (p<0.001), underexpressed IGF2BP2 with E2A/PBX1-positive (p<0.01), and overexpressed IGF2BP3 with MLL/AF4-positive (p<0.001) leukemias. In contrast to T-ALLs, B-ALLs negative for recurrent fusion genes underexpress IGF2BP2 (p<0.01) and overexpress IGF2BP3 (p<0.001) when compared to donor BM. Altogether, our results show that deregulation of normal IGF2BP expression pattern is associated with malignant B-lymphopoiesis. The potential utility of IGF2BP profiling in B-lymphoid neoplasms will emerge as the functions of IGF2BPs are further delineated.

This study was supported by the European Economic Area and Norwegian Financial Mechanism Grant No. 2004-LT0040-IP-1EEE.

Figure 1. Expression patterns of IGF2BPs.
**Hematopoiesis**

**0746**

C-CBL MEDIATES CYTOSKELETAL SIGNALS THROUGH RAC AND REGULATES INTERACTION OF IMMATURE HEMATOPOIETIC CELLS WITH THE BONE MARROW MICROENVIRONMENT

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**CELLS TRANSFORMED BY THE ACTIVE TYROSINE KINASE FUSION PROTEIN TEL/PDGFR BETA**

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Hematopoietic stem cells (HSC), have conceptual and practical importance in the field of stem cell expansion and differentiation for therapy purposes. Their self-renewal, proliferation and differentiation are controlled by a variety of intrinsic and extrinsic signals. In haematopoietic malignancies, the downregulation of c-Cbl and the leukaemic phenotype is still unclear. **Aims**. To determine the importance of intrinsically and extrinsically driven Wnt signalling in self-renewal and differentiation decisions using a LSC model. **Methods**. To address this we used a previously established and characterized tetracycline regulatable embryonic stem (ES) cell model to express the oncogene TEL/PDGFRbeta. Activation of the pathway was achieved via pharmacological GS3Kbeta inhibitors, Wnt3a stimulation or by over-expressing a dominant positive form of beta-catenin. Self renewal was assessed by alkaline phosphatase staining and TagMan Stem Cell Pluripotency Array. Haemopoietic commitment was assessed by flow cytometry and haemopoietic colony assays. **Results**. Expression of Tel/PDGFRbeta down-regulated the Wnt/GSK3beta/beta-catenin pathway and reduced ES cell self-renewal. Activation of the Wnt pathway substantially suppressed Tel/PDGFRbeta mediated differentiation, with Wnt3a stimulation, over-expression of beta-catenin and the GSK3beta inhibitor BIO all having this capacity. Analysis via the pluripotency array identified the gene signature involved in Tel/PDGFRbeta mediated early differentiation. As expected key self-renewal genes were down-regulated and key differentiation genes up-regulated by Tel/PDGFRbeta. Several of which are implicated in other malignancies the most interesting being; Flt3 an active tyrosine kinase involved in angiogenesis, HbG and Cdx2 which are also linked to leukaemic transformation. Activation of the canonical Wnt pathway either through expression of active beta-catenin or BIO treatment enhanced expression of self renewal/pluripotency genes down-regulated by Tel/PDGFRbeta (Nanog, Rex1, GDF5, sFRP2 & -K), whilst suppression of differentiation genes (Dkk1, Flt3, Pod1, Cdg9, Ocm1 & Gapa) was achieved. **Conclusions**. We clearly demonstrate that activation of the canonical Wnt pathway modulates the behaviour of LSC in our model, leading to enhanced self-renewal and suppressed differentiation. This has important implications as it demonstrates that the Wnt morphogens potentially play a greater role in sustaining LSC within the BM niche than previously perceived.

**0747**

CANONICAL WNT SIGNALLING MODULATES THE BEHAVIOUR OF STEM CELLS TRANSFORMED BY THE ACTIVE TYROSINE KINASE FUSION PROTEIN TEL/PDGFR BETA

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**Background**. Leukaemia is initiated by the transformation of a haemopoietic stem cell (HSC). Abrupt activation of the Wnt pathway has recently been linked to leukaemia through various mechanisms; autocrine up-regulation of Wnt morphogens, epigenetic silencing of negative regulatory molecules and activation of downstream signalling molecules. Although evidence supports a role for the canonical Wnt pathway in normal HSC self-renewal and haemopoiesis, how vital intrinsic and extrinsic signals are in sustaining leukaemic stem cells is still unclear. In the present study we investigated the potential role of Wnt signalling in the origin of leukaemia and the leukaemic phenotype is still unclear. **Aims**. To determine the importance of intrinsic and extrinsic Wnt signalling in self-renewal and differentiation decisions using a LSC model. **Methods**. To address this we utilised a previously established and characterized tetracycline regulatable embryonic stem (ES) cell model to express the oncogene Tel/PDGFRbeta. Activation of the pathway was achieved via pharmacological GS3Kbeta inhibitors, Wnt3a stimulation or by over-expressing a dominant positive form of beta-catenin. Self renewal was assessed by alkaline phosphatase staining and TagMan Stem Cell Pluripotency Array. Haemopoietic commitment was assessed by flow cytometry and haemopoietic colony assays. **Results**. Expression of Tel/PDGFRbeta down-regulated the Wnt/GSK3beta/beta-catenin pathway and reduced ES cell self-renewal. Activation of the Wnt pathway substantially suppressed Tel/PDGFRbeta mediated differentiation, with Wnt3a stimulation, over-expression of beta-catenin and the GSK3beta inhibitor BIO all having this capacity. Analysis via the pluripotency array identified the gene signature involved in Tel/PDGFRbeta mediated early differentiation. As expected key self-renewal genes were down-regulated and key differentiation genes up-regulated by Tel/PDGFRbeta. Several of which are implicated in other malignancies the most interesting being; Flt3 an active tyrosine kinase involved in angiogenesis, HbG and Cdx2 which are also linked to leukaemic transformation. Activation of the canonical Wnt pathway either through expression of active beta-catenin or BIO treatment enhanced expression of self renewal/pluripotency genes down-regulated by Tel/PDGFRbeta (Nanog, Rex1, GDF5, sFRP2 & -K), whilst suppression of differentiation genes (Dkk1, Flt3, Pod1, Cdg9, Ocm1 & Gapa) was achieved. **Conclusions**. We clearly demonstrate that activation of the canonical Wnt pathway modulates the behaviour of LSC in our model, leading to enhanced self-renewal and suppressed differentiation. This has important implications as it demonstrates that the Wnt morphogens potentially play a greater role in sustaining LSC within the BM niche than previously perceived.

**0748**

NOTCH REGULATES THE EXPRESSION OF HOXB4 AND GATA3 IN HEMATOPOIETIC STEM CELLS

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**Background**. The study of transcription factors (TF) and signaling pathways controlling processes involved in the biology of hematopoietic stem cells (HSCs), has conceptual and practical importance in the fields of stem cell expansion and differentiation for therapy purposes. In a previous study we showed that the transcript levels of the NF-kB subunits RELB and NFKB2, NOTCH1, HOXB4 and GATA3 were at higher levels in the more primitive CD133+ enriched HSC subpopulations, as compared to total CD34+ HSC. Moreover, transcript levels of NOTCH1 were highly correlated to those of HOXB4 and GATA3, and of NF-kB subunits RELB and NFKB2. In that study we investigated the potential regulation of GATA3 and HOXB4 by the Notch pathway, and its potential modulation by TNF-α, an activating-stimulus of the NF-kB pathway with known positive effects in T cell differentiation. **Methods**. Human CD34+ HSC were immunomagneti-
we have generated MxCre; ROSA-R25-EYFP mice and found that treat-
ment with both the chemotherapeutic agent 5-FU as well as the endo-
toxin LPS leads to the production of IFNA in HSCs. In addition, LPS
treatment in vivo induced a strong increase in proliferation of HSCs. To
our surprise, mice lacking the IFNA receptor still respond to LPS, indi-
cating that the induced proliferation of HSCs upon LPS treatment is in-
dependent of signaling via the IFNAR. In general, LPS will bind the
TLR4 CD14 receptor complex on cells, leading to the rosomal response
to LPS. When mice lacking TLR4 are treated with LPS, HSCs are no
longer activated in response to LPS, indicating that also the effect of LPS
on HSCs is dependent on TLR4 signaling. Interestingly, LPS induced ac-
tivation correlated with increased expression of Sca-1 on HSCs, similar
to the increased Sca-1 expression upon IFNA treatment. As for IFNA, the
up-regulation of Sca-1 is required for LPS induced proliferation, since Sca-
1 mice do not respond to LPS stimulation. In summary, these data sug-
gest that LPS induced bone marrow stress leads to the production of
IFNA in the bone marrow and increased proliferation of the HSCs. Thus,
in addition to viral infection also other forms of bone marrow stress, like
LPS, result in activation of dormant HSCs in the bone marrow. Further-
more, both IFNA and LPS induced activation of HSCs are dependent on
the up-regulation of Sca-1, suggesting a more general role for Sca-1 in
the activation of stem cells.

IGF2 differentially regulates haematopoiesis in fetal and adult haematopoietic stem and progenitor cells

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Background. The insulin-like growth factor (IGF) signalling pathway is important for proliferation and differentiation of haematopoietic stem cells (HSC) in mice. Activating mutations and/or aberrant IGF signalling are implicated in human cancers, including childhood leukemia and myeloma. The 2 principal ligands, IGF1 and IGF2, both signal through the same transmembrane receptor, IGF1R, while IGF2R negatively regulates IGF signalling by binding and internalising IGF2. Previous studies in mice suggest that IGF2 regulates fetal haematopoiesis and blocking IGF2/IGF1R signalling in human ES cells reduces their survival and clonogenicity. However, whether IGF1 and 2 are produced by fetal liver (FL) and bone marrow (BM) haematopoietic niches and their role in human fetal haematopoiesis is not known. Aims and Methods. To investigate this we assessed: i. IGF1/2 production by human FL and fetal BM MSC and fetal hepatocytes (FL cell line) ii. FL HSC and progenitor numbers and IGF expression and response compared to adult BM HSC/progenitors (MPP, LMPP, CMP, MEP, GMP and B-cell progenitors- BCP). Results. IGF2, as assessed by qRT-PCR, confirmed by Western blotting, was similarly highly expressed by FL, fetal BM and adult BM MSC (n=4 each) and human fetal hepatocytes. IGF1 was expressed by adult BM MSC but barely detectable in fetal MSC and hepatocytes. Flow cytometric analysis in conjunction with clonogenic and lymphoid differentiation assays showed that FL compared to adult BM CD34+ cells contained a 2-fold lower frequency of HSC and BCP but 2-4-fold higher frequency of MPP, LMPP, CMP, MEP and GMP. Consistent with a physiologic role for IGF2 in fetal haematopoiesis we found distinctly different patterns of IGF1R/IGF2R on fetal and adult BM CD34+ cells. In FL >95% of HSC and progenitors highly expressed IGF1R. IGF1R and IGF2R were highly co-expressed on HSC while fewer MPP, LMPP, CMP, MEP and GMP and BP co-expressed IGF2R with IGF1R and at considerably lower levels than HSC. In contrast to FL, fewer adult BM CD34+ cells expressed IGF1R and IGF2R and at much lower levels (MFI ~7-fold-3-fold less). The selective production of IGF2 by FL niche and differential expression pattern of IGF2R on FL CD34+ sub-populations is consistent with a modulatory role for IGF2-IGF2R interaction on these cells. Co-expression of high IGF2 levels with IGF1R on FL HSC may restrict HSC expansion hence the lower frequency of FL HSC while lower IGF2R in MPP and other progenitors would facilitate their expansion which may explain high MPP/HSC ratios in FL. In clonogenic assays IGF2, but not IGF1, increased clonogenicity of FL CD34+ cells. IGF2 caused marked stimulation of BFU-E and CFU-GM colonies and some CFU-GEMM and EPO-independent BFU-E. IGF1 caused modest stimulation of EPO-independent BFU-E at the expense of CFU-GM, although did not stimulate MK or CFU-GEMM suggesting differential downstream effects of IGF1R signalling in response to IGF2 compared to IGF1 in FL CD34+ cells. Conclusion. Together these data suggest a unique role for IGF2, rather than IGF1, in regulating normal fetal haematopoiesis in human embryos which may provide insight into the role of aberrant IGF signalling in childhood leukaemia, particularly in Down syndrome.
through heat-shock promoter, to establish stable germline transgenic zebrafish in order to study the influence of MYCN gene on the transcriptional regulation of hematopoiesis. Methods: Murine source MYCN cDNA was fused into the VEGF-tagged pSGH2 vector and was injected into zebrafish embryos at the one- or two-cell stage of development. These embryos were heat shocked in a 38°C incubator for 1 hour once between 14 to 19.5 hours post-fertilization (pf). MYCN-EGFP transgenic founder lines were identified on the basis of GFP expression at about 60 days pf. These founders were identified by fin clipping and genotyping, using PCR primers F:ATCACTGTGCGTCCCAAGA, R:TTAGCAAATGCAGGGCTTG. Then we established the stable germline transgenic zebrafish lines, to show its influence on hematopoietic regulatory factors through RT-PCR and the peripheral blood smear. Results. Eighteen of 256 (7.0%) mosaic F0 zebrafish embryos injected with the constructed vector were identified the germline transgenic zebrafish, including 8 male, 10 female. We extracted blood cells at 60 dpf from wild-type and the F1, F2 generation of transgenic fish. Using cytology, we determined that the blood cells from wild-type fish are predominantly erythrocytes, with myeloid cells only occasionally observed. By contrast, the blood cells from the transgenic fish contain abundant blast-like cells, which are larger than the erythrocytes and have high nuclear to cytoplasmic ratios. We extracted RNA from embryos of 1 dpf, 3 dpf, 7 dpf, and adult fish of 2 months pf, using RT-PCR, we found that MYCN expression remarkably results in scf (stem cells transcription factor, which is requirement in primitive hematopoiesis), mpo (myeloperoxidase; granulocyte specific gene) and gata1 (which is a master regulator in erythrocyte development) downregulation. Reversely, it up-regulates c-myb which is predominantly expressed in immature hematopoietic cells, and its expression decreases as these cells differentiate. Correspondingly, MYCN expression downregulates NDRG1(N-Myc downstream regulated gene 1, which is defined as differentiation related gene 1). Therefore, the blood phenotype induced by MYCN expression results in an accumulation of immature hematopoietic blast cells, significantly inhibiting erythropoiesis, and the differentiation of myelopoiesis. Summary. Zebrafish offers the advantages of high-throughput scale in the study of gene function in vivo. We report here the generation of a highly tractable model of MYCN expression, showing that induced expression of MYCN in zebrafish embryos results in rapid manifestation of a robust phenotype that exhibits cytological and transcriptional hallmarks of human hematopoietic malignancies (eg. AML), suggesting that MYCN signaling pathways are likely to be conserved between human and zebrafish. Most importantly, using this model enabled us to track the molecular changes that take place well before morphological phenotypes can be detected, and to determine the roles of candidate MYCN target genes. We demonstrate that MYCN regulates scf and several lineage-specific transcription factors, reprogramming hematopoietic cell fate in vivo.
osteo geneic differentiation was assessed at this stage. Clones were con-
sidered to have HPP if they reached confluent monolayer in 25 cm²
flask, if they grew to confluence in 6-well plate, were named
MCFC if reached confluence in 24-well plate and MMC if they didn’t
manage to cover the bottom of 24-well plate. MMSC clones with HPP
from 1 donor were analyzed by means of ligation-mediated poly-
merase chain reaction (LM-PCR). Results. It was shown that the propor-
tion of MMSC clones with HPP were 3.7 ± 0.6% at 1st passage, reached
maximum of 5.0 ± 1.4% at 3d passage and then declined. Clones with
HPP were not detected at 7th passage. The proportion of MMSC clones
with LPP reached maximum of 6 ± 2% by 2nd -3d passage and then
also declined. Such clones were not detected at 7th passage as well.
The maximum proportion of MCFC clones was 25 ± 12% at 5th pas-
sage and then declined to 7% at 7th passage. The majority of MMSC
clones possessed very low PP (MMC) and their mean proportion
through passages was 0.2 ± 4%. The vector integration sites were de-
termined in 10 out of 14 MMSC clones with HPP obtained from one
donor. Each analyzed clone contained unique integration sites. It means
that every analyzed clone was a progeny of distinct parental MMSC.
Clonal composition of MMSC was unique in each passage. The domi-
nant clones were not revealed. Thus not a single true mesenchymal
stem cell with the ability to self-renew was detected by LM-PCR
among studied clones. Conclusions. MMSCs represent a heterogeneous
population of cells with different but limited PP. The probability of the
presence of genuine mesenchymal stem cells in the population of
MMSCs seems to be very low.

### 0754

**PERSISTENT MICROCHIMERISM IN BONE MARROW: INFLUENCE OF MESENCHYMAL STEM CELLS**

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**Background.** Microchimerism in bone marrow (BM) can be detected
even years after allogeneic hematopoietic stem cell transplantation (Allo-HSCT), in spite of complete donor chimerism (CDC) attainment in peripheral blood (PB). Aims. The aim of our study was to explore the source of the persisting autologous feature in BM with particular in-
terest in mesenchymal stem cells (MSC), which are known to remain
of recipient origin after Allo-HSCT. Methods. BM cells obtained from
patients with CDC detected in PB (n=14; 5 after reduced intensity con-
ditioning, 6 after myeloablative conditioning; the median day post
Allo-HSCT= 1499) were cultured in cytokine-free medium, and MSC
were expanded. All clonal analyses were performed by real-time PCR ex-
ploring insertion/deletion polymorphism with the detection limit of at-
least 0.01%. Authenticity of MSC was affirmed morphologically and
by flow cytometry analysis (positivity for CD105, CD73, CD44, and
CD90 and negativity for the hematopoietic markers - CD14, CD19,
CD34, and CD45). Results. The proportion of autologous cells observed
in the whole BM was below 0.1% in all samples (see table). On the
other hand, analysis of cultivated MSC revealed their autologous
origin, as only a minimum of donor cells was detected (below 0.1% in
most cases). The amount of donor cells decreased with the number of per-
formed passages. Interestingly, the host origin of the MSC was also
identified in two samples with no autologous cells detected in whole
BM. Summary/Conclusions. Our results confirm and extend previous ob-
servations of the autologous origin of MSC. For the first time, we
clearly emphasize connection between MSC and persisting mi-
crochimerism in BM. The impact of MSC must be taken into account,
especially when danger of incipient relapse relating to microchimerism
analysis in BM is considered. Furthermore, undetected autologous cells
in BM do not signify even partial donor origin of MSC. e-mail:
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### 0755

**THERAPEUTIC POTENTIALS OF MESENCHYMAL STEM CELLS IN BONE DEFECTS, THE CASE STUDY IN RABBIT TIBIA**

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**Background.** Mesenchymal stem cells (MSC) have high proliferation
and differentiation capacity into other cell lineages. MSC could be used
in regenerative medicine because of these potentials. Distraction osteo-
genesis is the most prevalent therapy in bone defects. There are many
restricted situations in bone defects therapy such as collapsing of callus,
loss of lengthening and the long time of consolidation. Aims. The aim
of this study is to examine therapeutic potentials of mesenchymal stem
cells in bone defects. Methods. In this study, 21 New Zealand rabbit
were used. These rabbits were separated into control, stem cell and
osteoblast differentiated stem cells. Serum physiologic was applied to the
first group of rabbit, adipose-derived mesenchymal stem cells were in-
jected to callus site of the second group after distraction process to and
osteoblasts differentiated from mesenchymal stem cells were injected
to callus site of the third group of rabbits. Before injection, we charac-
terized stem cells by flow cytometry and the cells were tagged with
“green flourescent protein” (GFP). After four and eight weeks, the rab-
bis were sacrificed and evaluated radiologically, biomechanically and
histopathologicaly. Results. Radiological analyses revealed that callus
des parameter increased in Group III as compared to Group I and Group II. In biomechanical tests, the highest rates were
observed in Group III comparing to the others. As a result of
histopathological studies, it was also observed that the quality of
newly formed bone and the cells active in bone formation were signif-
icantly higher in Group III as compared to Group I and II rabbits. Sum-
mary/Conclusions. Taken together all these results revealed that os-
teoblasts differentiated from mesenchymal stem cells shortens the the
consolidation period of distraction osteogenesis. Stem cells can be used
effectively for the treatment of bone defects.

### 0756

**MYOFIBROBLASTS ORIGINATING FROM MYELOGENOUS LEUKEMIA CASES FORM BLASTOMA IN SEVERE COMBINED IMMUNODEFICIENCY MICE**

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**Background and Aims.** We recently reported that acute myelogenous
leukemia (AML) blasts as well as chronic myelogenous leukemia
(CML) cells convert to stromal myofibroblasts to create an environ-
ment for the proliferation of leukemic cells in vitro and in vivo. To ascer-
tain the biological characteristics of the bone marrow-derived myofi-
bromoblasts, myelogenous leukemia-derived stromal myofibroblasts were transplanted to NOD/SCID mice in vivo. Materials and Methods. The institutional ethical committee approved our study, and after obtaining informed consent bone marrow cells were collected from AML and CML patients as well as from informed normal individuals. Mononuclear cells were separated with gravity sedimentation method, and adherent cells were isolated by the two-hours-culture, and further culture of the population. During the culture, cells were split with trypsin/EDTA once a week. After two months the myofibroblasts were prepared, which characterized with flow cytometry to determine the expression of the specific proteins on their cell-surface. The prepared myofibroblasts (1 x 10^7 cells) were injected intravenously to two grey-rate NOD/SCID mice, and mice were bred in a pathogen-free room with antibiotics-containing water. Anti-TE671 antibody was injected every 11th day subcutaneously for the suppression of NK cells. Results and Discussion. Between day 40 and 60 after transplantation mice were dead. Autopsy findings revealed tumor formation at the liver. The transplanted myofibroblasts expressed STR-1, and smooth muscle actin but not lineage-specific molecules including CD56; however, bromoblast-forming cells expressed CD56 strongly but not other lineage-specific cell-surface markers. CD31, CD73, CD140a, and CD326(EpCAM) were also negative. These myofibroblast-derived bromoblasts expressed vascular endothelial growth factor (VEGF-A) and its receptor tyrosine 1, and 2, and showed growth promotion when cells were cultured with VEGF-A. These observation indicate that myeloid leukemia-derived myofibroblasts form bromo- blastoma in an autocrine-fashion of VEGF-A, and also that the CD56-positive specific fraction of myofibroblasts is selectively engrafted to form bromoblast. CD56 positive myofibroblast sarcoma of the liver is very rare disease that called Nested stromal epithelial tumor of the liver. Here we can reproduce this tumor in NOD/SCID mouse system, and we think CD56 positive fraction of myofibroblasts are its origin. Further study will reveal its precise biological characteristics.

0757
ACCELERATED TELOMERE SHORTENING IS AN EARLY AND PERMANENT SIGNATURE IN CULTURED HUMAN MESENCHYMAL STEM CELLS EXPOSED TO CHEMOTHERAPY

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Background. Induction of cell senescence has been shown to be produced by various agents, including chemotherapy drugs. However, the mechanisms involved in the ageing pathway, in particular the decrease of telomere sequences, for up to 28 days in culture. ATM phosphorylation was documented early after Doxo and Eto exposure, while no senescence was observed. Following a single drug exposure, MSC were unable to regain the telomere length potential.

Methods. MSC were exposed to sub-lethal doses of drugs able to induce double-stranded DNA breaks, i.e. doxorubicin (Doxo), etoposide (Eto), and mice were bred in a pathogen-free room with antibiotics-containing water. Anti-TE671 antibody was injected every 11th day subcutaneously for the suppression of NK cells. Results and Discussion. Between day 40 and 60 after transplantation mice were dead. Autopsy findings revealed tumor formation at the liver. The transplanted myofibroblasts expressed STR-1, and smooth muscle actin but not lineage-specific molecules including CD56; however, bromoblast-forming cells expressed CD56 strongly but not other lineage-specific cell-surface markers. CD31, CD73, CD140a, and CD326(EpCAM) were also negative. These myofibroblast-derived bromoblasts expressed vascular endothelial growth factor (VEGF-A) and its receptor tyrosine 1, and 2, and showed growth promotion when cells were cultured with VEGF-A. These observation indicate that myeloid leukemia-derived myofibroblasts form bromoblastoma in an autocrine-fashion of VEGF-A, and also that the CD56-positive specific fraction of myofibroblasts is selectively engrafted to form bromoblastoma. CD56 positive myofibroblast sarcoma of the liver is very rare disease that called Nested stromal epithelial tumor of the liver. Here we can reproduce this tumor in NOD/SCID mouse system, and we think CD56 positive fraction of myofibroblasts are its origin. Further study will reveal its precise biological characteristics.

0758
CD146 AS A MARKER FOR CHARACTERISATION OF MSC POPULATIONS

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It is often assumed that expression of a certain broad set of markers defines various types of cell cultures as MSCs, but in reality, most of these markers are expressed by cultures of fibroblastic cells from any tissue. In addition, most of these markers are highly modulated in culture, which makes it difficult to characterize stromal cell cultures. In human BM, CD146 marks adventitial reticular cells a classicaly known as a poor phenotype residing in a subendothelial position over the abluminar surface of BM sinusoids. In other tissues, CD146 is expressed by pericytes, the cell that are considered as the MSCs found in different tissues. This study was designed to find out if CD146 could be used as a surrogate marker for characterization of MSC populations. Patients and Methods. 30 donors without bone marrow disorders were involved in this study. MSC cultures from BM (BM-MSC) were evaluated in successive passages (P) for immunophenotype (FACS analysis), frequency of colony-forming units (CFU-F) in MSC population, frequency of adipo- and osteo-progenitors (CFU-Ad, CFU-Ost) in the same population and for dynamic of changes in these properties with successive passages. CD31+, CD105+, CD90+ and CD73+ of MSC were studied by limiting dilution assay followed by induction of adipo-or osteo-differentiation: cell suspension was serially diluted two folds across the 8 columns of 96-well plates, resulting in columns containing from 50 to 0,59 cells per well. After 10 days of culture the number of positive and negative wells was counted. Normality for each cell concentration and CFU frequency was calculated. Then plates were induced to undergo adipogenesis and osteogenesis and CFU-Ad was determined by Oil Red staining and CFU-Ost by Alizarin Red staining after 14 and 21 days respectively. All calculations were performed as for CFU-F; β-galactosidase activity was used as a biomarker for assessing replicative senescence; growth rate was estimated using on-line calculator www.doubling-time.com. Results. In present study we have found that, as expected, MSC at all passages were positive for stromal cell markers CD105/90/73 and negative for hematopoietic markers CD34/19/45. The level of expression of stromal markers remain stable at all passages. However, while the population of CD146+ cells was abundant in MSC at early passages, this population declined with successive passages (P4 vs 14+ 6% at P5). This decline in CD146+ population correlates with decline in frequency of CFU-F (30,6%+4,9% at P1 vs 8,0%+1,5% at P4), CFU-Ost (17%+4,9% at P1 vs 5%+2% at P4) and CFU-Ad (10%+2% at P4 vs 3%+1% at P4), in MSC sample. Additionally, while CD146+ population decreased in MSC sample, population doubling time increased dramatically and β-galactosidase activity increased. Conclusion. The population of CD146 positive MSCs declined with subsequent passages and correlated with the decrease of the ability to differentiate to Ost and Ad and increase of population senescence. The level of CD146 expression could be used to predict some of the properties of BM-MSC sample.
from 329 (290 full term and 39 preterm) healthy newborns with gestational age between 38-41 and 29-37 weeks respectively, delivered either vaginally or by cesarean section, were prospectively studied. Among preterm infants 4/39 were twins. Gestational age was determined from the last menstrual period and early ultrasonography. Blood samples were collected from the umbilical vein in tubes with EDTA K3, immediately after delivery and were analyzed within 3 hours. The hematological variables were determined in automated counter Sysmex XE2100 (Japan). Reference values were performed according to non-parametric percentile method (CLSI C28-A3). Results. Higher NRBC count was recorded in preterm newborn infants regardless from the type of delivery. Modest correlation was obtained between IRF and the RET count, but significantly different from zero. The data verified that the number of circulating RET corresponds to a greater fraction of young reticulocytes. Reference values in UCB in relation to gestational age for term infants (n=290) and preterm infants (n=39), respectively: 

1. Young reticulocytes. Reference values in UCB in relation to gestational age for term infants (n=290) and preterm infants (n=39), respectively:

   - IRF% 18.0223-47.288 (n=288) and 0.080-5.550 (n=39), RET 106 u/L 0.093-0.244
   - NRBC% 0.00-20.508 (n=290) and 0.600-50.400 (n=39), NRBC 103 /uL

   Conclusion. It is important to define reference values for the interpretation of blood count in the neonatal period. Our data showed decreased NRBC count with advancing gestation. We underline the possible value of high NRBCs in UCB suggesting an intrauterine hypoxic event several hours before birth. The IRF seems to have an independent role as an early and sensitive marker of erythropoietic activity and is becoming helpful in routine laboratory practice.

0760

**CTL4 POLYMORPHISMS AND MICROENVIRONMENT IN PEDIATRIC CLASSICAL HODGKIN LYMPHOMA. CLUES FOR THE UNDERSTANDING OF THE SUPPRESSOR PHENOTYP**

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Background. Classical Hodgkin lymphoma (cHL) is characterized by a low percentage of Hodgkin and Reed-Stemberg cells amidst a high fraction of infiltrating inflammatory cells, mostly CD4+ lymphocytes with a suppressor profile. The cytotoxic T-lymphocyte antigen-4 (CTLA4) is an immunoreceptor that inhibits T-cell proliferation and activation. Its polymorphisms appear to differentially influence its inhibitory activity. Particularly, the CTLA4 +49GC and CT60GC genotypes, and the CT60GC/+49GC haplotype were associated with a low inhibitory potential and with the susceptibility to autoimmune diseases. The inverse model was proposed for cancer, where genetic variants with high inhibitory potential (CT60A/+49A) would negatively influence anti-tumor immune surveillance. Thus, a better clinical response would be expected in the presence of genetic variants with increased anti-tumor capacity (low inhibitory potential). Aims. To investigate if CTLA4 polymorphisms influence the tumor microenvironment composition, clinical characteristics and outcome in cHL. Methods. 100 children (3 to 18y, median 15) diagnosed with cHL were included. DNA was extracted from peripheral blood or tumor diagnostic samples. Two CTLA4 polymorphisms (+49A/G and CT60A/G) were evaluated by TaqMan allele discrimination assays (ABI7000). CD4, T-bet (Th1), c-maf (Th2), CD8, Granzyme-B, Tia-1, FoxP3, CD20 and Ki67 expression were evaluated by immunohistochemistry in tissue microarray (TMA) slides. EBV was detected by EBER-ISH. Results. The presence of the CTLA4+49A allele was associated with high numbers of T lymphocytes (>451/mm2, percentile 25) (p=0.033). Genetic variants with high inhibitory potential (CT60A allele and +49A/CT60A haplotypes) were associated with high numbers of CD4+ lymphocytes (p=0.025), which was secondary to an increase of Th2 lymphocytes (C-maf+), as compared to Th1 (T-bet+) lymphocytes (p=0.046). CT60 low inhibitory potential variants (CT60G allele and GG genotype) were related with higher numbers of CD8+ (p=0.014) and FoxP3+ lymphocytes (p=0.007). These results suggested that while CTLA4 polymorphisms may influence the activation status and proliferation of Treg cells, the CD8+ lymphocytes sub-population appears to be a target for the inhibitory effect imparted by another cell population(s) expressing high CTLA4 levels. A better event-free survival (EFS) was associated with the CTLA4+49AA genotype (p=0.045, Log-rank test), while a worst EFS was observed in cases with CTLA4 haplotype 49G/CT60G (p=0.015). Differences in EFS were more marked in the EBV+ group, suggesting that FoxP3+ lymphocytes might be engaged in the immune response against EBV antigens. In the Cox regression adjusted by EBV status, haplotype 49G/CT60G maintained its independent prognostic impact (p=0.043; HR CI95% 0.08-0.95). Conclusions. This is the first study to investigate CTLA4 polymorphisms in cHL and shows a potential effect of CTLA4 variants on cHL microenvironment, and outcome. In the pediatric cHL, high inhibitory potential CTLA4 variants were not associated with worse anti-tumor responses, but to favorable microenvironmental characteristics and better therapeutic responses. This reinforces the existence of a distinct anti-tumor model of immune response in hematopoietic malignancies as compared to solid cancers and highlights, through a genetic approach, the suppressor nature of the immune response against classical Hodgkin lymphoma.

0761

**T(4;8)(Q27;Q24) IN HODGKIN LYMPHOMA CELLS TARGETS PHOSPHODIESTERASE PDE5A AND HOMEBOX GENE ZHX2, ACTIVATING STAT1-SIGNALING**

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Background. Hodgkin/Reed-Sternberg (HRS) cells represent the malignant fraction of infiltrated lymph nodes in Hodgkin lymphoma (HL). Although HRS cells display multiple chromosomal aberrations, few are
recurrent and the targeted genes unknown. Aims. Cell line L-1236 was used as a model to specify recurrent chromosomal breakpoints in HL. Methods. Fluorescence in situ hybridization (FISH), genomic copy number analysis by Agilent 4x180K-arrays, quantitative real-time PCR (QF-PCR), Western blot, Immuno-cytochemistry, RNAi-mediated knockdown, expression profiling by Affymetrix U133A arrays. Results. Here, we analyzed the karyotype of HL cell line L-1236 by multicolor FISH and identified multiple abnormalities, including t(4;8)(q27;q24). We focused on this alteration because both breakpoint regions (4q2 and 8q24) are recurrently involved in HL. Mapping these breakpoints was performed by FISH using tilepath BAC clones together with high density genomic arrays, while expression analysis of candidate target genes was performed by QF-PCR and Western blot. The data revealed activation of phosphodiesterase PDE5A at 4q27 and activation of homeobox gene ZHX2 at 8q24. This breakpoint is located in the far upstream region of ZHX2, thereby removing potential binding sites for transcription factors XBP1 and MSX1. Expression analysis of ZHX2 and of promoter-constructs demonstrated ZHX2 activation by XBP1, and repression by MSX1 in combination with corepressor histone H1C. L-1236 cells showed silencing of XBP1 and elevated coexpression of MSX1 and H1C, associated with chromosomal alterations of the histone gene cluster at 6p22. Conspicuous expression of XBP1 in SUP-HD1 HL cells coincided with downstream deletions and enhanced ZHX2 levels. Together confirming the activating role of XBP1, while highlighting an alternative mode of aberrant ZHX2 expression. To identify ZHX2 target genes we performed expression profiling of L-1236 cells following ZHX2 knockdown by siRNA treatment. Our data revealed suppression of STAT1 and several interferon-beta-targets, suggesting aberrant activation of STAT1-signaling by ZHX2. ZHX2-mediated STAT1-overexpression was confirmed in L-1236 cells by QF-PCR and Western blot. Additionally, elevated expression of STAT1 protein was detected in all analyzed HL cell lines in contrast to control B-cell lines, highlighting a general feature of HL. Interestingly, treatment of L-1236 with PDE5A-inhibitor Sildenafil inhibited STAT1-phosphorylation and -signaling, indicating synergistic activity of the t(4;8)-target genes PDE5A and ZHX2. Conclusion. Taken together, we have identified a novel aberration in HL, t(4;8)(q27;q24), activating PDE5A and ZHX2. Both genes promote STAT1-signaling, which is generally elevated in HL cells and may contribute to the malignant phenotype. Furthermore, our data suggest that phosphodiesterase-inhibitors may represent a promising therapeutic avenue in HL.

0762
A LOW PERIPHERAL BLOOD CD4/CD8 T CELLS RATIO PREDICTS POOR PROGNOSIS IN CLASSICAL HODGKIN’S LYMPHOMA
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Background. Classical Hodgkin’s lymphoma (cHL) is characterized by the presence of neoplastic cells in a rich background of T and B cells, macrophages and other inflammatory cells. The contribution of these non tumor cells to the pathogenesis of HL is still poorly understood; T cells constitute a significant component of the reactive infiltrate in cHL with an elevated T helper/T suppressor (CD4/CD8) ratio. Our aim was to evaluate the prognostic significance of a low peripheral blood (PB) CD4/CD8 cells ratio at diagnosis in cHL patients (pts).

Methods. Flowcytometry immunophenotyping of PB samples was performed at diagnosis in 92 immunocompetent pts (with or without radiotherapy (RT)) treated at our institution between January 2007 and December 2008. Median age at diagnosis was 34 years (range 15-62), 42 pts (45.6%) were male, 50 pts (55.6%) presented advanced stage disease (IIIB-IV), 36 (39%) bulky disease, 40 (43.6%) had B symptoms, 24 (26%) spleen involvement, 25 (27.1%) extranodal disease. The histology was nodular sclerosis in 79 (85.9%), mixed cellularity in 13 (14.1%). Results. A PB CD4/CD8 T cells ratio <1.5 (Low CD4/CD8 ratio) was recorded in 40 pts (45.5%). The clinical features present with a higher rate in the low CD4/CD8 T cells ratio group were: nodular sclerosis histology (96% vs 77%; p<0.05), high bulky disease (70.5% vs 45.7%; p<0.05), lymph node involvement (51.8% vs 35.7%; p<0.05), bulky disease (51.8% vs 28.7% p<0.05), spleen involvement (40.7% vs 14.3% p<0.05). At a median follow-up of 24 months, 15 (16.3%) pts developed progressive/relapsed disease; 70% of these patients had a CD4/CD8 t cells ratio <1.5. The incidence of positive PET after two courses of chemotherapy was higher in pts with a low CD4/CD8 t cells ratio (40.7% vs. 17.14%; p<0.05). The 24 months progression free survival (PFS) rate was 76.2% in low CD4/CD8 t cells ratio pts and 92.2% in patients with a CD4/CD8 t cells ratio >1.5 (p<0.05). No significant differences were observed for sex, absolute lymphocyte count, VES, LDH and extranodal disease between the low and high CD4/CD8 ratio groups. Conclusion. A low PB CD4/CD8 t cells ratio at diagnosis seems to be associated with unfavorable clinical features and a worse prognosis. Further investigations including analysis of t reg, cytokine, chemokine, are needed to confirm these results.

0763
BASELINE SERUM C-REACTIVE PROTEIN LEVELS (CRP) IN HODGKIN LYMPHOMA (HL): CORRELATIONS WITH CLINICAL AND LABORATORY PARAMETERS AND PROGNOSTIC SIGNIFICANCE UNDER ANTHRACYCLINE-BASED CHEMOTHERAPY
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Background. Serum CRP levels are elevated in the majority of patients with HL at diagnosis, reflecting tumor burden and aggressive biologic behavior. Despite being an easily and frequently measured marker, data on its potential prognostic significance are extremely limited. Aims. To analyze the correlation between baseline CRP levels and clinical-laboratory findings and outcome of patients with HL treated with anthracycline-based chemotherapy with or without radiotherapy (RT).

Methods. Baseline CRP levels were recorded in 496 patients with HL, who were treated with anthracycline-based chemotherapy with or without radiotherapy (RT) in 2 Centers. Baseline CRP levels were correlated with other baseline clinical-laboratory features (Spearman correlation coefficient) and the disease behavior. Discriminant analysis was used to classify CRP levels, which were then subjected to Kaplan-Meier survival curves.

Results. The median value of CRP levels in the 496 evaluable patients was 21.10 mg/L; 27% and
73% had normal and elevated CRP levels respectively. Baseline CRP levels correlated with virtually all other parameters reflecting tumor burden or disease aggressiveness: There were strong correlations with advanced stages and B-symptoms (p<0.001) as well as specific extranodal sites (bone marrow, lung, liver). Baseline CRP presented a very strong correlation with ESR (Spearman’s rho 0.74, p<0.001), strong correlation with hemoglobin (inversely), platelet counts and lymphocytes (inversely) (S-rho 0.40-0.60, p<0.001) and looser correlations with white blood cell counts, serum LDH, and absolute lymphocyte counts (inverse) (S-rho <0.35, p<0.001). A trend towards a dose-response effect with FFS was observed: 5-year FFS for patients with CRP levels <5, 5-21, 21-69, and ≥70 mg/L (roughly the CRP quartiles) was 81%, 78%, 68% and 66% (p=0.09). The difference was more pronounced for patients with normal versus elevated CRP levels (81% versus 71%, p=0.006). Differences in OS were not significant (p>0.10). The prognostic impact of CRP on FFS was borderline in early stage patients (IIA, IIB: 5-year FFS 83% versus 73% for normal versus elevated levels, p=0.09), but no difference was detected in advanced disease (68% versus 66%, p=0.48). CRP was an independent adverse prognostic factor for FFS, when adjusted for stage (III/IV versus I/I), B-symptoms and IPS factors (except of lymphocytopenia). However, baseline CRP was not analyzed along with ESR in the same model, because of their very strong correlation, raising issues of collinearity. Conclusions. CRP levels close to cut-off levels in ~70% of HL patients at diagnosis and correlated with FFS independently from other established prognostic factors included in the IPS. The very strong correlation between baseline CRP and ESR probably suggests that only one of them should be included in a given prognostic model. Even larger patient series should be analyzed in order to draw final conclusions, but the final selection might be relied on specific biologic and technical advantages of either marker.

**0765**

LYMPHOCYTE IMPAIRMENT IS AN INDEPENDENT FACTOR USEFUL TO PREDICT INTERIM-PET ASSESSMENT IN HODGKIN’S LYMPHOMA PATIENTS

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Background. Lymphoid suppression plays a key role in determining the tumor growth and progression in Hodgkin’s Lymphoma (HL) and this inflammatory dysregulation seems to be linked with the interim-PET assessment. CD62L reduction on the surface of lymphocytes represents a marker of immune-suppression. Aim. In the present work, we evaluated the CD62L expression on lymphocytes surfaces in HL patients at diagnosis and related these findings with the interim-PET outcome. Methods. 27 HL patients, 18 at stage I-II and 9 at stage III-IV, treated with ABVD as first line, were enrolled and PET assessment was performed at diagnosis and after two cycles (interim-PET). PET images were interpreted visually according to Dann et al., 2010. 2 out of 18 patients (11.1 %) at early stage and 5 out of 9 (55%) at stage III-IV had a positive interim-PET. CD62L has been evaluated from peripheral blood at diagnosis on CD4 and CD8 T-lymphocytes and expressed as intensity mean fluorescence (IFM). T-test has been performed in order to assess if there is a statistical difference in CD62L levels between different groups. Pearson correlation has been used to define the relationship between CD62L expression and other clinical-laboratoristic parameters. Receiver-operating-characteristic (ROC) curves has been used in order to determine cut-off levels. Informed consensus has been signed. Results. We observed reduced levels of CD62L expression on CD8+ lymphocytes surface between HL patients at diagnosis and healthy controls matched for gender and age (57.2 vs 42.45) (p=0.007), but not on CD4+ lymphocytes (average 62.4 vs 61.7) (p=0.85). No statistical differences have been found in CD62L expression on CD4+ and CD8+ lymphocytes at early stage versus advanced stage (p=0.06 for CD62L on CD4+ and p=0.8 for CD62L on CD8+). Interestingly, CD62L expression levels on CD8+ cells was not related with interim-PET assessment in any stage, whereas CD62L reduction on CD4+ lymphocytes related with the interim-PET assessment only in patients at early stage, being in patients with interim-PET negative higher than in patients with interim-PET positive (average 68.69 vs 54) (p=0.03). Additionally, CD62L expression on both CD8+ and CD4+ lymphocytes did not relate with prognostic and inflammatory factors. Conclusion. On the whole, our findings suggest that HL patients have a reduced expression of CD62L on CD8 compared to healthy controls and patients at early stages who have also a reduction of CD62L on CD4+ lymphocytes have a poorer prognosis.
Background. Published data regarding the effect of advanced age on the outcome of patients with HL are contradictory. Reported differences in prognosis between older and younger patients may have been due to differences in treatment intensity, inadequate staging, inferior efficacy of salvage therapy and presence of comorbid conditions. Aims. To compare the clinical and laboratory features and the outcome of patients with HL ≥60 years treated with anthracycline-based chemotherapy (CT) or combined modality therapy with the subgroup of younger patients. Methods. Patients ≥60 years old, who were diagnosed with HL and treated with anthracycline-based CT+RT in our Department, were evaluated and compared with younger patients for their demographic, laboratory features and outcome. Results. More subgroups were further subdivided in 3 age groups: 60-66, 67-74, ≥75 years old, which were also compared. Results. Among 1004 patients with HL treated with anthracycline-based CT+RT, 113 (11%) were ≥60 years old. When compared with younger patients, they had a higher incidence of advanced disease (55% vs 45%, p=0.03). Several potential adverse prognostic features were significantly more prevalent in older patients, including B-symptoms (p=0.01), bone marrow (p=0.005) and liver (p=0.001) involvement, low serum albumin (p=0.003) and elevated β2-microglobulin levels (p<0.001). IPS was clearly higher in older patients (p=0.001). In contrast, lung involvement (p=0.004) and significant leukocytosis (p=0.057) were more prevalent in the younger age group. This probably reflected the much higher incidence of nodular sclerosis in younger patients (70% vs 41%) with corresponding lower incidence of mixed cellularity (19% vs 44%) (p=0.001). The 10-year failure free survival (FFS) was not significantly different in older patients compared to young ones (76% vs 76%, p=0.76). However, older patients had a higher incidence of deaths during treatment (0.2% vs 4.4%, p<0.001), inferior 10-year overall survival (86% vs 46%, p<0.0001), HL-specific survival (91% vs 73%, p<0.0001) and 5-year survival after failure (62% vs 14%, p<0.0001). Among the 113 patients aged ≥60 years, 47, 45 and 21 were 60-66, 67-74 and ≥75 years old respectively. These subgroups had similar baseline characteristics with the exception of worsening anaemia and more β2-microglobulin elevations with age. However, deaths during treatment were 0%, 6.7% and 9.5%, respectively (p=0.14), while the 5-year survival after failure was 27%, 0% and 0% respectively (p=0.0008). No significant differences were observed for FFS, while OS and HL-specific survival were marginally inferior in more advanced age groups. Summary/Conclusions. Despite differences in baseline clinical and laboratory features, patients with HL ≥60 years old have similar failure rates with younger ones when treated with anthracycline-based CT+RT. However, the higher incidence of deaths during treatment and the poor results of salvage therapy resulted to inferior overall and HL-specific survival rates. Within the ≥60 years group, the latter problems were pronounced in more advanced age subgroups.

Background. Hodgkin’s disease (HD) is the most common non-AIDS defining tumour diagnosed in HIV setting. The introduction of highly active antiretroviral therapy (HAART) has opened a new prospective in the treatment of pts with HD-HIV since the better control of the underlying HIV infection allows the use of more aggressive chemotherapy regimens, including high dose chemotherapy. However, up to now prognostic factors on overall survival (OS) or time to treatment failure (TTF) have not yet been identified. Methods. In order to identify prognostic factors, we analyzed data on 596 pts with HD-HIV diagnosed and treated in 90 different Institutions from 5 European countries from October 1995 to March 2010. All factors were analyzed for OS and TTF. Results. 86% of pts were male and the median CD4 cell count was 224/dl (range 3-1274); 52% of pts had mixed cellularity subtype, stages III-IV were diagnosed in 72% of cases and 55% of pts had extranodal involvement (bone marrow 35%, spleen 21%, liver 14%). Conclusion. We identified a new “European Score” for HD-HIV able to predict different outcomes in these patients. This score should be considered for future prospective studies.

Background. Despite differences in baseline clinical and laboratory features, patients with HL ≥60 years old have similar failure rates with younger ones when treated with anthracycline-based chemotherapy (CT) or combined modality therapy with the subgroup of younger patients. Methods. Patients ≥60 years old, who were diagnosed with HL and treated with anthracycline-based CT+RT in our Department, were evaluated and compared with younger patients for their demographic, laboratory features and outcome. Results. Among 1004 patients with HL treated with anthracycline-based CT+RT, 113 (11%) were ≥60 years old. When compared with younger patients, they had a higher incidence of advanced disease (55% vs 45%, p=0.03). Several potential adverse prognostic features were significantly more prevalent in older patients, including B-symptoms (p=0.01), bone marrow (p=0.005) and liver (p=0.001) involvement, low serum albumin (p=0.003) and elevated β2-microglobulin levels (p<0.001). IPS was clearly higher in older patients (p=0.001). In contrast, lung involvement (p=0.004) and significant leukocytosis (p=0.057) were more prevalent in the younger age group. This probably reflected the much higher incidence of nodular sclerosis in younger patients (70% vs 41%) with corresponding lower incidence of mixed cellularity (19% vs 44%) (p=0.001). The 10-year failure free survival (FFS) was not significantly different in older patients compared to young ones (76% vs 76%, p=0.76). However, older patients had a higher incidence of deaths during treatment (0.2% vs 4.4%, p<0.001), inferior 10-year overall survival (86% vs 46%, p<0.0001), HL-specific survival (91% vs 73%, p<0.0001) and 5-year survival after failure (62% vs 14%, p<0.0001). Among the 113 patients aged ≥60 years, 47, 45 and 21 were 60-66, 67-74 and ≥75 years old respectively. These subgroups had similar baseline characteristics with the exception of worsening anaemia and more β2-microglobulin elevations with age. However, deaths during treatment were 0%, 6.7% and 9.5%, respectively (p=0.14), while the 5-year survival after failure was 27%, 0% and 0% respectively (p=0.0008). No significant differences were observed for FFS, while OS and HL-specific survival were marginally inferior in more advanced age groups. Summary/Conclusions. Despite differences in baseline clinical and laboratory features, patients with HL ≥60 years old have similar failure rates with younger ones when treated with anthracycline-based CT+RT. However, the higher incidence of deaths during treatment and the poor results of salvage therapy resulted to inferior overall and HL-specific survival rates. Within the ≥60 years group, the latter problems were pronounced in more advanced age subgroups.

Background. Hodgkin’s disease (HD) is a malignant diseases with the highest rate of cure particularly if diagnosed in early stage. Neverthe-
The importance of immature myeloid cells in the prognostic assessment of Hodgkin lymphoma

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Background. In Hodgkin lymphoma (HL), the interim PET (after 2 cycles of ABVD chemotherapy) is the most important prognostic factor and it is probably linked to the persistence of the reactive microenvironment that promotes tumor cell survival. CD68+ tumor-associated macrophages (TAM) are increased in cases with poor prognosis, thus to be proposed as additional prognostic marker at diagnosis of HL. Their progenitors circulating in peripheral blood are well known in solid tumors as Myeloid Derived Suppressor Cells (MDSC) for their ability to suppress T-cell immune responses. In mice, MDSC are defined as being Gr1+CD11b+ cells capable of suppressing antigen-specific or suppress T-cell immune responses. In mice, MDSC are broadly defined and it is probably linked to the persistence of the reactive microenvironment of ABVD chemotherapy. We assessed the expression of CD11b+ MDSC and their correlation with clinical findings, interim-PET response and treatment outcome, and T-cell subpopulations, including Treg (CD4+CD25+FoxP3+).

Results. Flow cytometry and a negative PET-PET performed after two cycles of ABVD chemotherapy in pts with localized Hodgkin's disease were evaluated. Bulky disease pts were the object of our analysis. FDG-PET was performed after two cycles (PET2) was positive in 18/79 pts (23%); 8 (44%) progressed or relapsed and 10 maintained CR. By contrast 53/60 (88%) pts with a negative PET2 remained in CR. Thus the positive predictive value of a PET2 in bulky disease was very low (44%) and the negative predictive value was 88%. The specificity and sensitivity of PET2 were 58% and 84%, respectively. Radiotherapy was performed in 17 PET2 positive pts, 10 did not fail (59%), one pt with PET2 positivity did not perform radiotherapy and progressed. A FDG-PET was performed at the end of therapeutic program (PET6), all pts (10) with positive PET2 and 5/6 with negative PET2 progressed. In univariate analysis negative FDG-PET performed after two cycles (p=0.002) and in particular negative PET2 (<0.0001) were statistically correlated with a better progression free survival. With a median follow-up of 37 months (range 4-103) 73 pts are alive and 63 (81%) are free from progression. The 2-yr PFS probability for PET2 negative and for PET2 positive patients were 97% and 40% respectively (p=0.002). Conclusions. With the IHP interpretation criteria we observed a large number of false positive PET2 in mediastinal bulky early stage Hodgkin disease. For this reason new PET evaluation methods in this subset of pts are mandatory. Moreover in bulky disease pts radiotherapy could permit to overcome the poor prognostic significant. A prospective multicenter study confirms that a negative PET-PET performed after two courses of conventional standard dose therapy in localized bulky disease pts was able to predict a favourable outcome.

0771 ‘EARLY FDG-PET’ PREDICTS CLINICAL COURSE OF HODGKIN’S LYMPHOMA ALTHOUGH DOES NOT CORRELATE WITH MACROPHAGES INFLTRATION IN DIAGNOSTIC SPECIMENS

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Background. Hodgkin’s lymphoma (HL) is a highly curable malignancy that mostly affects young adults; however despite satisfactory results, about 20% of patients still die of relapsed/refractory disease and late toxic effects rate, often due to overtreatment, continue to rise with time. Consequently, the optimal treatment should be designed based on prognostic models, but currently all of them predict the outcome with imperfect accuracy. Since early FDG-PET and more recently tissue macrophages infiltration in diagnostic specimens emerged as powerful prognostic predictors, we hypothesized that macrophage infiltration could be the shadow of the inflammatory microenvironment, that FDG-PET identifies in HL and that persists at early assessment in patients who will fail the treatment. Aims. The primary end point of this study was to verify the prognostic role both of “early-FDG PET” and of macrophage infiltration, while the secondary endpoint was to test if “early-FDG PET” positivity could correlate with high macrophage infiltration in diagnostic specimens. Methods. A homogeneous cohort of fifty-two patients (M/F: 28/24; median age 35 yrs) was diagnosed and treated at Siena and Florence hematology departments between February 2007 and July 2010 was extracted from local databases and retrospectively analyzed. All patients had completed staging with whole body CT scan, FDG-PET and bone marrow biopsy. One patient had stage I, 20 pts. Stage II, 24 stage III and 7 stage IV. Treatment plans...
5-grade scale (see below).

Study Group (GHSG) definition. PET-2 was evaluated using the established 5-grade scale (see below).

Results. Median age of the patients was 28 years (19-72), 69% were men, 77% had nodular sclerosis, 3, 10 and 13 had stage II, III and IV respectively, and 70% had B-symptoms. The background. Persistent PET positivity following 2 cycles ABVD (PET-2) is a strong unfavorable prognostic factor in advanced HL. Progression Free Survival is approximately 95% vs 15% for patients with negative vs positive PET-2 respectively. The evaluation of PET-2 is based on two defined, arbitrary criteria, which require prospective validation. Whether PET-2 based early treatment modification can improve prognosis is unknown. However, it is known that the continuation of ABVD after a positive PET-2 is associated with an unacceptably high risk of treatment failure. Aims. (1) To analyze patients with advanced stage HL who were evaluated by PET-2 after ABVD x2 in relation to the final response; (2) To analyze the impact of PET-2 on subsequent treatment plan; (3) To make an attempt to validate current criteria for PET-2 positivity. Patients and Methods. We present a retrospective study of 26 patients with advanced stage HL who were evaluated by PET-2 after ABVD x2 in relation to the final response.

Peto et al. Blood 2002;100:1984-1988). The median follow-up is 67 months (range 3-156 months) The 5-year overall survival (OS), freedom from progression (FFP), disease free survival and event free survival are 54%, 52%, 60% and 37%, respectively. The 5-year OS is significantly different in pts with an international prognostic score (IPS) >2 in comparison to that of pts with an IPS <3 (4% vs 36%, p = 0.0005). Similarly, the percentages of FFP at 5 years in these groups are 72% and 45% (p = 0.03). Conclusions. Our data confirm the long term efficacy of Stanford V regimen in combination with HAART in HD-HIV. However, Stanford V is significantly less effective in pts with IPS>2 and therefore new strategies be tested in this setting. Supported by AIRC and ISS grants.

0774

INTERIM FDG-PET SCANNING IN PATIENTS WITH HODGKIN LYMPHOMA AND HIV PREDICT RESPONSE TO ABVD CHEMOTHERAPY

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Background. There is approximately a 10-fold increase in the incidence of Hodgkin lymphoma (HL) in patients with Human Immunodeficiency Virus (HIV) and patients often present with advanced disease. A number of studies have demonstrated a good outcome after ABVD (doxorubicin, bleomycin, vinblastine and dacarbazine) chemotherapy combined with highly active antiretroviral therapy (HAART) in patients with HIV-HL. There is still much debate whether more aggressive regimens such as escalated BEACOPP are more appropriate in patients with advanced disease. The prognostic significance of interim PET in HIV negative patients with HL is now well established. However, there is no published data regarding interim PET scanning in pa-
tients with HL in the HIV setting. Aims. Our objective was to evaluate the role of interim FDG-PET imaging during ABVD chemotherapy in determining response assessment for patients receiving first-line treatment for advanced HIV-HL and to assess the outcome in such patients.

Patients and Methods. 21 patients with HIV-HL from five UK centres treated with ABVD and concomitant HAART were included. Interim PET at cycles 2 or 3 of ABVD (PET-2 or PET-3) was carried out in all patients. At the time of therapy, the median age was 42 (range 32-60; 18 M: 3 F). Results. Median CD4 count at diagnosis of HL was 187 cells/µl (range 33-995 cells/µl) and majority of patients (76%) had an undetectable plasma HIV viral load (VL). All patients presented with either advanced stage disease (n=18) or stage 2a disease with adverse prognostic factors (n=3). Just under half (48%) presented with stage IV disease. All cases were of classical HL and histologic subtypes included: mixed cellularity 8 (38%), nodular sclerosis 7 (33%), unknown 6 (29%). Median Hasenclever IPS was 3 (range 1-6). Of 21 evaluated patients, 20 achieved complete remission (CR) after first-line therapy. Interim PET was negative in 86% (18/21) of patients. Treatment failure was seen in 1 of the 3 interim PET positive patients and none of the 18 interim PET negative patients. The 2-yr progression free survival for patients with a positive interim PET was 67% and for negative interim PET was 100% (p = 0.012). After a median follow up of 20 months (range 5-45), all patients are in continued complete remission (1 after second treatment; 15 after third-line therapy for relapse). Cox’s multivariate findings suggest a negative interim PET maybe highly predictive of successful treatment outcome in Hodgkin’s lymphoma even in the setting of HIV disease.

0776
THE PROGNOSTIC SIGNIFICANCE OF SPECIFIC ORGAN INVOLVEMENT AND THE NUMBER OF INVOLVED EXTRANODAL SITES IN ADVANCED STAGE HODGKIN’S LYMPHOMA
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Background. Extranodal dissemination has independent prognostic value within advanced stage Hodgkin lymphoma (HL). It remains controversial whether a specific organ involvement and the number of involved extranodal (EN) sites are prognostically unfavorable. Aims. To analyze the prognostic value of specific organ involvement and number of involved EN sites in advanced stage classical HL patients (pts). Methods. A cohort of 100 advanced stage classical HL pts treated with ABVD (1997-2005) were analyzed for the prognostic relevance of bone marrow, lungs and liver involvement, as well as two or more involved EN sites. The median follow up was 7 years. Their significance was tested according to response rate an overall survival (OS). Results. The distribution of EN dissemination was: 28 pts with bone marrow involvement, the lungs involved in 14 pts, the liver involved in 12 pts and other sites involved in 4 pts. Two or more EN localizations were found in 14 pts. Complete remission rate was significantly lower in pts with liver involvement (50% vs. 83%, p=0.017). A shorter OS was associated with bone marrow (p=0.005), lungs (p=0.022) and liver (p=0.005) involvement. Further, two or more EN sites had adverse effect on OS (p=0.000). Lung involvement and EN two or more localizations had adverse effect on event free survival (EFS) (p=0.000; p=0.001), respectively. Cox’s multivariate model revealed EN two or more localizations as a significant independent prognostic factor for OS (p=0.000) and lungs involvement for EFS (p=0.000). Summary/Conclusions. Advanced stage classical HL pts with EN two or more localization and lungs involvement are at higher risk of treatment failure and might be eligible for more effective treatment approach.

0777
OUTCOME AND PROGNOSTIC FACTORS IN HODGKIN LYMPHOMA (HL) PATIENTS WHO RELAPSE AFTER HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION (HDT/ASCT)
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Background. Patients with HL who relapse after HDT/ASCT are considered incurable. Aims. To evaluate the outcome and prognostic factors of 45 HL patients, who relapsed after HDT/ASCT. Methods. Out of 95 consecutive patients with primary refractory/relapsed HL undergoing HDT/ASCT at a single unit from March 1996 to January 2010, 45 patients who relapsed after HDT/ASCT were recorded. Thirty-one out of 45 (69%) were males, their median age at ASCT was 28 years (19-54), 40 (89%) underwent HDT/ASCT at first relapse, 5 (10%) for multiple relapses and 3 (6%) for primary refractory disease. Prior to HDT/ASCT, 14 (31%) were in complete remission (CR), 18 (40%) in partial remission (PR) and 13 (29%) were chemoresistant. Results. Median time between ASCT and the following relapse was 6.3 months (1-54). At relapse after ASCT median hemoglobin was 11.5 g/dl (7.6-14.4), 32%, 18%, 24% KAS 26% had clinical stage I, II, III kov 1 respectively and 27% had B symptoms. The median number of involved sites was 3 (1-12), 16% had bulky and 41% extranodal disease. The most common involved extranodal sites were lungs, bones and soft tissue.
Patients received the following therapies: 47% radiotherapy (RT), 25% chemotherapy containing gemcitabine/vinorelbine +/- liposomal doxorubicin, and 22% MOPP-like chemotherapy. Furthermore, 5 patients underwent a second ASCT and 2 patients allogeneic stem cell transplantation. At a median follow up of 36 months, 3-year freedom from second failure (FF2F) and 3-year overall survival (OS) after relapse was 23% vs 64%, respectively. Thirteen out of 45 patients died of HL, 4 due to disease progression, 6 due to non-hematological complications, 15 are alive with active HL, and 13 are alive in CR. Prognostic factor analysis for FF2F disclosed that a short (<12 months) interval between ASCT and relapse (p<0.002), chemoresistance prior to ASCT (p<0.002), anemia (p<0.004) and B symptoms at relapse post ASCT (p<0.02) were of unfavorable prognostic significance. Chemoresistance prior to ASCT (p<0.005), anemia (p<0.03) and B symptoms at relapse (p<0.0001) were unfavorable prognostic parameters for OS, as well. In additional, age >45 (p<0.03) proved as a poor prognostic factor for OS. Despite the limited number of patients, multivariate analysis revealed that chemoresistance prior to ASCT and B symptoms at relapse post ASCT were independent prognostic factors for FF2F and OS.

Conclusions. Patients with HL who relapse after ASCT have a poor prognosis. However, the natural history of the disease remains long, with 2/3 of the patients remaining alive at 5 years after relapse. Chemoresistance prior to ASCT, B symptoms and anemia at relapse post ASCT, as well as, relapse in <12 months after ASCT, are unfavorable prognostic parameters.

**Table 1. Efficacy and toxicity rate.**

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In the group of historical control ORR was 89.39%; that is significantly lower than in both studied groups (p<0.05). The therapy in 4 patients in the group of BEACOPP-14 and 6 patients in the group of BEACOPP-esc was changed to ABVD according to the toxicity. The most frequent toxicity type in both groups was hematological toxicity of different grades (72.8% in the group of BEACOPP-14 and 67.6 in the group of BEACOPP-esc, p<0.05). The special feature of BEACOPP-14 was a significantly higher rate of anemia (25%) compared to 12.5% in the group of BEACOPP-esc. In 7.5% the BEACOPP-14 cycles were not completed due to neutropenia of 4th grade. In 1 patient in the group of BEACOPP-esc died because of infectious complication. Non-hematological toxicity was observed in 45.6% cycles of BEACOPP-14 and in 81.5% cycles of BEACOPP-esc. The most frequent nonhematological complications were nausea and vomiting (Table). Conclusion. The therapy efficacy in both groups of BEACOPP-14 and BEACOPP-esc was higher than in the group of historical control (treatment with ABVD). Both comparative regimens show almost equal treatment efficacy and toxicity rates in patients with HD of the poor prognosis group. However, the results are preliminary and should be confirmed in larger number of patients and with a longer follow-up.

**0778**

**BEACOPP-14 VS. BEACOPP-ESC IN PATIENTS WITH HODGKIN’S DISEASE FROM POOR-PREDICTION GROUP: INTERIM ANALYSIS OF PROSPECTIVE RANDOMIZED MULTICENTER STUDY**

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**Aim.** To compare the efficacy and toxicity of the treatment with B EACOPP-14 and B EACOPP-esc regimens in patients with Hodgkin’s disease (HD) from high risk group in prospective randomized study.

**Methods.** Since September 2008 103 patients in 6 Ukrainian centers from 18 to 65 years old (median 29 years), 48 male and 55 female with bulky disease; 7% stage III-IV) homogeneously treated with 4-6 cycles ABVD and not receiving pelvic radiotherapy. At diagnosis no patients had disorders of the menstrual cycle. Twenty-two patients received therapy with MOPP/ABVD/GnRH-a vs MOPP/ABVD p= 0.04). GnRH-a decreased gonadotoxic effect (amenorrhea) of high-risk chemotherapy (MOPP/ABVD/GnRH-a vs MOPP/ABVD p= 0.04). Results. Maximal observation period in both groups was 29 months. Overall response rate (ORR) after the completion of the treatment was 97.9% in the group of BEACOPP-14 and 98.1% in the group of BEACOPP-esc; p>0.05 (Table). In the group of historical control ORR was 89.39%; that is significantly lower than in both studied groups; p<0.05. The therapy in 4 patients in the group of BEACOPP-14 and 6 patients in the group of BEACOPP-esc was changed to ABVD according to the toxicity. The most frequent toxicity type in both groups was hematological toxicity of different grades (72.8% in the group of BEACOPP-14 and 67.6 in the group of BEACOPP-esc, p<0.05). The special feature of BEACOPP-14 was higher rate of anemia: 25% compared to 12.5% in the group of BEACOPP-esc. In 7.5% the BEACOPP-14 cycles were not completed due to neutropenia of 4th grade. In 1 patient in the group of BEACOPP-esc died because of infectious complication. Non-hematological toxicity was observed in 45.6% cycles of BEACOPP-14 and in 81.5% cycles of BEACOPP-esc. The most frequent nonhematological complications were nausea and vomiting (Table). Conclusion. The therapy efficacy in both groups of BEACOPP-14 and B EACOPP-esc was higher than in the group of historical control (treatment with ABVD). Both comparative regimens show almost equal treatment efficacy and toxicity rates in patients with HD of the poor prognosis group. However, the results are preliminary and should be confirmed in larger number of patients and with a longer follow-up.

**GnRH-ANALOGOUS IN YOUNG PATIENTS AFFECTED BY HODGKIN DISEASE (HD) TREATED WITH ABVD**

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**Background.** The mechanism by which chemotherapy causes long-term gonadotoxic effect is not completely understood. Few data are available on features predicting infertility risk and gonadotoxic dysfunction. Despite there aren’t standardized methods evaluating fertility in this subset of patients, resumption of the menstrual cycle is considered as evidence of an ovarian function. This resumption represents a desirable goal in long-term survivals of treated Hodgkin’s Lymphoma, in order to protect from unfavorable effects of premature amenorrhea. There are currently no approved procedures certainly effective in trying to preserve fertility (ovarian suppression by treatment with estrogenic or gonadotropin-releasing hormone agonist analogue, ovum or ovarian tissue cryopreservation). The gonadic function in patients with HD seems to be adversely affected by therapies containing alkylating agents or procarbazine, by employed doses (BEACOPP and BEACOPP esc), by advanced stage disease and by age. Conversely there is no evidence on the gonadotoxic effect of ABVD protocol. Matter under discussion is the induction of ovarian suppression by administration of GnRH analogue (GnRH-a) in order to reduce the risk of ovarian failure associated with chemotherapy. Aims. Aim of this study was to evaluate the gonadotoxic potential (High/Intermediate/Low/No risk) of ABVD protocol and to examine the ovarian reserve after chemotherapy in a group of patients with HD treated according to protocol ABVD alone or in combination with GnRH-a, defining as primary end-point recovery and regularity of menstrual cycles. Methods. In our group of patients the incidence of amenorrhea after ABVD protocol was 1/51. The analysis for the event amenorrhea between the groups GnRH-a +/- showed no significant differences; the analysis conducted for the events irregularity and regularity of menstrual cycles showed differences within the limits of statistical significance between groups GnRH-a +/- (p = 0.07). Significant was the assessment of the relative risk: the amenorrhea RR 4.16 (CI 1.6-8.9), irregular cycles RR 6.5 (CI 1.1-8.9) and regular cycles RR 0.52 (0.1-4.9). ABVD group vs historical group had lower risk for amenorrhea (p=0.01) and GnRH-a decreased gonadotoxic effect (amenorrhea) of high-risk chemotherapy (MOPP/ABVD/GnRH-a vs MOPP/ABVD p= 0.04). Con-
ABVD protocol. These data suggest low-intermediate gonadotoxic potential of ABVD protocol and indicate a protective effect (RR>1) of inhibition of the hypothalamic-pituitary axis by the administration of GnRH-a on the risk of amenorrhea and irregular menstrual cycles after treatment with ABVD protocol.

**0780**

**SALVAGE THERAPY FOR RELAPSING OR RESISTANT Hodgkin LYMPHOMA: RETROSPECTIVE ANALYSIS OF RESULTS UTILIZING THREE DIFFERENT DEBULKING REGIMENS INCLUDING DHAP, IGEV OR IGEV FOLLOWED BY ESCALATED BEACOPP**

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**Aims.** To retrospectively evaluate our experience with different debulking regimens including DHAP, IGEV and IGEV followed by escalated BEACOPP. Patients and Methods. We reviewed 90 patients treated from April 1998 to December 2009 for resistant or relapsed Hodgkin lymphoma. Second-line chemotherapy changed over-time from DHAP x 2 cycles (group A) to IGEV x 1 followed by escalated BEACOPP x 2 (group B) and IGEV x 4 cycles (group C). Patient features are illustrated in the Table.

**Table 1.**

Overall, first-line CT was ABVD in 83 and MOPP alternated to ABVD in 7 patients. Involved-field RT had been given in 82 patients. PBSCT mobilization was carried-out after the first cycle of DHAP or the first cycle of IGEV, both in group B and C. Survival was calculated with the actuarial method. Results. The overall response rate (ORR) of group A was 57% (CR in 8 of 28 pts: 29%), of group B 82% (CR in 15 of 23 pts: 65%) and of group C 77% (CR in 11 of 59 pts: 28%). No patient failed to mobilize PBSCT; the median number of harvested CD34+ cells after DHAP (group A) was 11.8 x 10^6/Kg (range: 2.6-48), and after IGEV (groups B and C) 12.3 x 10^6/Kg (range: 3.7-50.8). Overall, 81 patients underwent PBSCT (25 in group A, 21 in group B, 35 in group C); 34 of them (42%) were given additional chemotherapy before BEAM conditioning: 14 (50%) in group A, 4 (17%) in group B, 16 (41%) in group C. The 2-yr PFS was 85%, 56% and 84% in groups A, B and C; in the same groups, the 5-yr overall survival was 81%, 75% and 72%, respectively. Conclusions. The retrospective nature of this survey does not allow firm conclusions on the best second-line therapy in Hodgkin lymphoma. Altogether, the three different approaches allowed an overall response rate of 72%, with a 2-yr PFS of 42%, and no difference in the 5-yr overall survival between the three groups of therapy was observed. In our experience, both IGEV and DHAP showed a good mobilizing capacity, while the inclusion of escalated BEACOPP produced a better CR rate compared to DHAP and IGEV and a higher 2-yr PFS rate.
Immune thrombocytopenia and other platelet disorders

**0782**
**DETERMINANTS OF PERSISTING THROMBOCYTOPENIA IN PATIENTS WITH TYPE 1 GAUCHER DISEASE TREATED WITH IMIGLUCERASE FOR 4-5 YEARS**

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Background. Thrombocytopenia in type 1 Gaucher disease (GD1) may result in surgical, obstetrical and spontaneous bleeding. Thrombocytopenia generally responds rapidly to imiglucerase enzyme replacement therapy but, in rare cases, the platelet response seems to be delayed and thrombocytopenia persists. Aim. To identify patient characteristics associated with (and potentially predictive of) persisting thrombocytopenia despite therapy. Methods. Patient characteristics associated with persisting thrombocytopenia were investigated by retrospective analysis of data from the International Gaucher Group (ICGG) Registry. A total of 1,016 GD1 patients with an intact spleen were classified into four groups by last platelet counts were known and who received continuous imiglucerase therapy for 4-5 years, were classified into four groups by last platelet counts were known and who received continuous imiglucerase therapy for 4-5 years. Possible associations with persisting thrombocytopenia after 4-5 years of therapy. Correlations of the study and at day 90. CBC was done for all patients every 3 days till day 90. Results. The mean baseline platelet count was 31x10^3 /microl. (range 21x10^3 - 42x10^3). Eleven of the 12 studied patients showed a highly significant (p value 0.000) increase in the platelet count with a maximum peak of 220x10^3/microl., occurring between days 15-33 in most patients. These patients proceeded to their planned surgery which included cataract, hemia and fracture fixation. No postoperative bleeding complications were recorded. There was no change in the reticulin grade in bone marrow of any of the patients. There were no significant changes in liver function but there was a significant increase in the bilirubin level. One patient did not respond by platelet count increase during the time schedule of the study and hence did not undergo surgical intervention. Conclusion. Romiplostim can be used under close follow up in chronic hepatitis C patients with liver cirrhosis and severe thrombocytopenia preoperatively in a dose of 2 mg/kg once weekly for four weeks. Additional studies are necessary to define the optimal dose and schedule of romiplostim.

**0783**
**PREOPERATIVE USE OF ROMIPLOSTIM IN THROMBOCYTOPENIC PATIENTS WITH CHRONIC HEPATIS C AND LIVER CIRRHOSIS**

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Background. Romiplostim is a thrombopoietin mimetic “pepti-body” comprising a human immunoglobulin IgG1 Fc domain covalently linked at each of its two C-terminals to two 14-amino-acid peptides that bind to and stimulate the thrombopoietin receptor. Continuous treatment with Romiplostim increases platelet counts in patients with immune thrombocytopenia for up to 5 years, with few adverse effects. Hepatitis C virus (HCV) represents the second most common blood-borne illness in the world, affecting up to 2% of the world’s population. Egypt reports the highest prevalence of HCV worldwide, ranging from 6% to more than 40% with an average of 13.8%. Thrombocytopenia, usually from Hypersplenism (and possibly from altered thrombopoietin metabolism or antiplatelet antibodies), is common in patients with cirrhosis. Aim. To detect the efficacy of Romiplostim use in thrombocytopenic patients with chronic hepatitis C and liver cirrhosis preoperatively. Methods. Our study was performed on 12 patients in the Electricity Hospital, Cairo, Egypt, having chronic liver disease with liver cirrhosis and they were classified as Child-Pugh score C with thrombocytopenia. All the patients started Romiplostim injections at a dose of 2 mcg/kg once weekly for four weeks. CBC, liver and kidney function tests, bone marrow aspirate and biopsy were done to all patients at start of the study and at day 90. CBC was done for all patients every 3 days till day 90. Results. The mean baseline platelet count was 31x10^3 /microl. (range 21x10^3 - 42x10^3). Eleven of the 12 studied patients showed a highly significant (p value 0.000) increase in the platelet count with a maximum peak of 220x10^3/microl., occurring between days 15-33 in most patients. These patients proceeded to their planned surgery which included cataract, hemia and fracture fixation. No postoperative bleeding complications were recorded. There was no change in the reticulin grade in bone marrow of any of the patients. There were no significant changes in liver function but there was a significant increase in the bilirubin level. One patient did not respond by platelet count increase during the time schedule of the study and hence did not undergo surgical intervention. Conclusion. Romiplostim can be used under close follow up in chronic hepatitis C patients with liver cirrhosis and severe thrombocytopenia preoperatively in a dose of 2 mcg/kg once weekly for four weeks. Additional studies are necessary to define the optimal dose and schedule of romiplostim.

Figure 1. Platelet count in patients.
0784
PRESENCE OF ADAMTS13 AUTOANTIBODIES IN OBESE SUBJECTS AS A POSSIBLE LINK BETWEEN OBESITY AND THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background. Thrombotic thrombocytopenic purpura (TTP) is a rare but often fatal disorder, whose mechanisms are yet to be fully understood. It is characterized by widespread thrombosis in the arterioles and capillaries of multiple organs and is mainly associated with severe deficiency of ADAMTS13, a disintegrin and metalloprotease with thrombospondin (TSP)-1 repeats. This enzyme cleaves high molecular weight von Willebrand Factor1 (ULWVF) multimers freshly released from activated endothelial cells to smaller and less active forms. Recently it has been reported that morbid obesity represents a risk factor for TTP, but the possible mechanisms underlying the phenomenon are still unknown. In obesity a low grade chronic inflammation state exists, with increased levels of adipokines, among them thrombospondin-1 (TSP-1). Aims. We aimed to investigate possible mechanisms linking obesity and risk of developing TTP. We also tried to understand the role of TSP-1 as a possible causative factor of TTP in obesity, given its structural homology with ADAMTS13 and its high expression by adipose tissue. Methods. 80 obese and 39 lean subjects were characterized by anthropometric, metabolic and inflammatory parameters and compared to 52 patients with TTP in clinical remission. ADAMTS13 antigen and activity, ADAMTS13 autoantibodies, TSP-1 and various cytokines’ levels were measured. Results. 21.3% of obese patients had a positive titre of non-inhibitory ADAMTS13 autoantibodies, while all lean subjects were negative. TSP-1 levels were significantly higher in obese and patients with TTP than in lean subjects. TSP-1 levels in obese patients were inversely correlated with ADAMTS13 activity. Moreover anti-ADAMTS13 antibodies cross-reacted with TSP-1 in obese subjects and patients with TTP in clinical remission. Conclusions. The presence of non-inhibitory anti-ADAMTS13 autoantibodies in some obese subjects may be induced by increased TSP-1 levels. Autoantibodies are probably directed against TSP-1 and cross-react with ADAMTS13 thrombospondin-domain. Over time somatic hypermutations could generate inhibitory activity, leading to an acute episode of TTP.

0785
MARKED VARIABILITY IN PLATELET RESPONSE TO ASPIRIN IN HEALTHY INDIVIDUALS: A CROSSOVER STUDY OF PLATELET FUNCTION TESTS

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Background. The Antiplatlet Trialists’ Collaboration reported a 25% reduction in death, MI, CVA in high-risk patients treated with aspirin. ‘Aspirin resistance’ describes recurrent events in aspirin-treated patients with reported rates between 0.4 and 83% depending on the assay used. This study assessed aspirin response in healthy individuals and the performance of various assays. Aims. This study assessed the prevalence of aspirin resistance in a cohort of healthy individuals. In addition, the performance of various platelet function tests was examined. A genetic substudy was also performed. Methods. A repeated measures, crossover trial was performed in healthy aspirin-naïve subjects aged 18-60 years. Written informed consent was obtained. Ethical approval was granted by the Office for Research Ethics Committee NI (ORECNI). The study was funded by Royal Hospitals Trust, Belfast and Northern Ireland Chest Heart and Stroke Association (NICHSA). Subjects were randomised to aspirin dose (75mg or 300mg) and sequence (ABBA, BAAB, ABAB, BABA, A=Aspirin, B=Placebo). The study consisted of 4-week treatment periods. Testing (Optical Platelet Aggregation, PFA-100, VerifyNow, serum TXB2 and urinary 11-dTXB2) was performed at baseline and at the end of each treatment period. Standard definitions of aspirin resistance were used. OPA (AA 0.5) was deemed the ‘gold standard’ (max aggregation 20%). Compliance was deemed satisfactory at interview. Statistical analysis was performed using Windows SPSS17. Results. The overall rate of suboptimal aspirin response was assay-dependant and varied greatly from 2.4% (OPA AA) to 63.5% (OPA ADP). Only 3 subjects were ‘aspirin resistant’ (via OPA AA) on all occasions. Overall sensitivities ranged from 27.5% (OPA ADP) to 87.5% (serum TXB2). Overall specificities ranged from 85.4% (VerifyNow) to 95% (serum TXB2). In addition, selection of alternative ‘cut-off’ values chosen for the PFA-100, OPA ADP and serum TXB2 produced marked variation in the calculated prevalence, sensitivities and specificities of these assays. No association was found between aspirin resistance and genetic polymorphisms. Summary/Conclusions. Response to aspirin shows significant inter- and intra-individual, inter-assay and temporal variability. The overall prevalence of aspirin resistance ranged from 2.4 to 63.5% depending on assay selection. Testing on multiple occasions using several assays is necessary to reliably predict aspirin response. Selection of alternative ‘cut-off’ values alters assay performance and caution should be used before categorising patients as ‘aspirin resistant’.

0786
ENHANCED P2Y12 INHIBITION IN PATIENTS WITH PREVIOUSLY LOW CLOPIDOGREL RESPONSE AFTER DOUBLING THE DOSE

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Aims. Residual platelet reactivity evaluated by platelet function tests is associated with adverse cardiovascular events in patients at risk on antiplatelet therapy. We compared the in-vitro efficacy of antiplatelet treatment in patients with double and standard dose of clopidogrel assessing vasodilator phosphoprotein (VASP) P2Y12 specific assay and light transmission aggregometry (LTA). Methods. We retrospectively evaluated a cohort of 461 patients by calculating the platelet reactivity index (PRI) based on the VASP assay and by ADP-induced platelet aggregation (ADP max, using 5 µM ADP). Among the patients, 390 were under dual antiplatelet therapy with acetylsalicylic acid and standard dose clopidogrel. A total of 129 patients (33.1%) showed a PRI >79, indicating a low response to clopidogrel. Results. Overall PRI and ADP max were 60 ± 41 % and 52 ± 36 %, respectively. There was no significant difference in PRI and ADP max between standard and double dose clopidogrel (student t-test p<0.5). Patients with low clopidogrel response under standard dose revealed PRI and ADP max of 83 ±14% and 61 ± 24%, respectively. In patients with previously poor clopidogrel response, treatment with double dose clopidogrel resulted in significantly lower PRI 60 ± 35% (n=41, student t-test p<0.0001), whereas ADP max showed only a trend to significance (see figure) (ADP max 54 ± 33%, p=0.06). Conclusions. In accordance with previous data, we observed no significant difference in an overall cohort of patients between standard and double dose clopidogrel. However, for patients with low clopidogrel response, doubling the dose enhanced the P2Y12 inhibition assessed by VASP and LTA.

Figure 1. Reduction in platelet reactivity with double dose.
were less than 60 x 10^9/L or if there was a previous diagnosis of pri-
was conducted. The diagnosis of ITP was established if platelet counts
during pregnancy or with a history of immune thrombocytopenia (ITP)
mated platelet count 5 had a platelet count less than 70 x 10^9/L and may
count was determined in 8 women at delivery. According to the auto-
quired treatment. Using the immunoplatelet count all patients had
pregnancy and 5 women in the late stages of pregnancy would have re-
results of therapy (median 10, range 4-23). Results: Overall, 27 patients achieved a complete re-
CR, 65.8%), 5 a partial response (PR, 12.2%) and 7 (17%) did not respond. Response rates were similar among the different no-
subgroups (CR: AIHA 80%, ITP 64.7%, TTP 75%, other 100%, PR: AIHA 25%, ITP 11.8%, TTP 12.5%). Response rates were similar
standard deviations among patients of all ages and in the median age 45 years, range 16-84 years) with various non-malignant hemato-
lc subgroups (CR: AIHA 80%, ITP 64.7%, TTP 75%, other 100%, PR: AIHA 25%, ITP 11.8%, TTP 12.5%).
5 women had anti-HCV(+) and additional 2 with ITP out of 5 tested, were H.Py-
for those with ITP, 24.8 months for those with TTP and 24.8 months for patients with other diseases. Overall, 10 of the 32 responded patients (31.3%) re-
them were retreated with rituximab alone (2) or in association with pulses of high dose dexamethasone (5). Six of these patients responded again to rit-
steroids and achieved a second CR (4) or a PR (2). After a median follow-up of 30 months (range 1.1-110 months) 39 patients are alive, 31 of them in CR, 6 in PR and 2 have active uncontrolled disease. Two patients have died due to disease-related complications. Conclu-
sions. Rituximab alone or in combination with corticosteroids is an ef-
effective second-line treatment for patients with autoimmune hemato-
ological disorders at a smaller dose than that administered to patients with lymphoproliferative disorders.

**0789**

**ASSOCIATION OF PLATELET INDICES WITH THYROID-STIMULATING HORMONE AND THYROID HORMONES IN EUTHYROIDIC HEALTHY SUBJECTS**

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**Background.** Platelet parameters and especially mean platelet volume, an important determinant of platelet function and morphology, consti-
tutes a novel emerging risk factor for atherosclerosis and its complica-
tions such as coronary heart disease. Mean platelet volume (MPV), platelet distribution width (PDW), platelet count and their correlations
thyroid-stimulating hormone (TSH), triiodothyronine (T3) and tri-
odothyronine (T3) have not been studied in depth. Aim. The aim of this study was to explore the correlation of platelet indices with TSH and thyroid hormones in euthyroidic healthy subjects. Methods. We have evaluated 82 euthyroidic healthy subjects (65 women and 19 men) with a mean age: 58 years (range: 22-78 years). None of the sub-
jects had infectious and neoplastic conditions, diabetes mellitus, hyper-
tension and dyslipidemia. To assess thrombopoeisis, we have deter-
mined platelet indices using Sysmex 9000 analyzer. TSH, free-T3 and free-T4 were determined using electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyzer (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with SPSS® version 17 for Windows software. Pearson or Spearman corre-
lation coefficients (r) were used for normally or not normally distrib-
uted variables respectively. Results. From the statistical analysis of the
data, the following results emerged: 1) There was a significant negative correlation between TSH and MPV in euthyroidic subjects (Spearman r=-0.747, p<0.001). 2) There was a significant negative correlation be-
tween TSH and PDW (Spearman r=-0.818, p<0.001, and 3) No statisti-
cally significant associations emerged between free-T3, free-T4 and
platelet parameters (p>0.05). In a multiple linear regression model, con-
trolling for age, gender, body mass index and thyroid hormones, serum TSH is a statistically significant predictor of MPV and PDW levels (p<0.05) in euthyroid healthy subjects. Conclusion. Our results indicate that TSH levels play an important predictive role in platelet markers which reflect platelet morphology and function. These results may also suggest that individuals with lower TSH levels tend to present increased platelet activation which could contribute to an increased risk of atherothrombotic complications in the future.  

**0790**

**PLATELET PARAMETERS IN PATIENTS WITH SUBCLINICAL HYPERTHYROIDISM: A CROSS-SECTIONAL STUDY**

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Background. Subclinical hyperthyroidism (SCH) is often encountered in the general population. Patients may be asymptomatic or may present non-specific symptoms. It has been suggested that SCH could be a risk factor for cardiovascular disease. On the other hand, platelet parameters and especially mean platelet volume, an important determinant of platelet function and morphology, constitutes a novel emerging risk factor for atherosclerosis and its complications such as coronary heart disease. Increased mean platelet volume (MPV) reflects active and large platelets that could release more thromboxane than smaller ones. MPV, platelet distribution width (PDW), platelet count (PLT) have not been studied in depth in subclinical hyperthyroidism. Aim. The aim of the present study is to compare the platelet count as well as the platelet parameters MPV and PDW in subclinical hyperthyroidism and in euthyroid healthy subjects and to investigate whether SCH may have a predictive significance in the determination of platelet size. Methods. In a cross-sectional study between 2007 and 2010, we have evaluated thirty five patients with subclinical hyperthyroidism prior to any therapeutic intervention (26 women and 9 men) with a mean age: 32.5 ± 7.3 years) and year/month of diagnosis (±1 month). None of the subjects (patients and controls) presented any infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. To assess thrombopoiesis, we have determined platelet indices using Sysmex 9000 analyser. Thrombostimulating hormone, thrombomodulin (T3), free-T3, thyroxine (T4) and free-T4 were determined using an electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyser (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with SPSS® version 17 for Windows software. Results. Cases presented significantly higher MPV (mean ± SD: 12.27 fl ± 0.46) and PDW (mean ± SD: 15.3 ± 1) than controls (mean MPV ± SD: 10.65 fl ± 0.78, p<0.001 and mean PDW ± SD: 13.5 ± 1.34, p<0.001). On the contrary, patients with subclinical hyperthyroidism had similar number of platelets per mm3 than healthy euthyroid subjects (mean PLT in patients: 253 x 109/mm3 ± 34 versus mean PLT in controls 264 x 109/mm3 ± 38, p=0.15). In a linear regression model, adjusting for age, gender, body mass index and smoking status, the presence of subclinical hyperthyroidism was the most significant predictor of MPV and PDW levels (p<0.001). Conclusion. These results suggest that subjects with subclinical hyperthyroidism tend to present increased platelet size and activation. Elevated platelet activation could contribute to an increased risk of atherothrombotic complications observed in SCH. Finally, these findings suggest that platelet morphologic changes observed in subclinical hyperthyroidism, such as higher MPV and PDW could be attributed to metabolic parameters.

**0791**

**HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN ADULT SERBIAN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA (ITP) PURPURA**

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Background. Multiple available treatments in ITP are aimed at minimizing the risk of bleeding and increasing the patients' platelets. However, these treatments beside bleeding may impact on patients' HRQoL. Aim: To evaluate the effect of ITP and its treatment on the patients’ HRQoL. Methods. Short form SF-36 health Survey was used to measure HRQoL; it was analyzed referring to the most important demographic and clinical characteristics in multivariate regression analysis. Hamilton tests were used for the assessment of anxiety and depression. Results. A total of 48/111 (43.2%) patients reported a history of transfusions for the treatment of ITP. At the time of the survey the median platelet count was 76x109/L, range 3-500x109/L; bleeding was present in 18 (16.3%) patients; 44 (39.6%) patients received some therapy: 31.8% corticosteroids, splenectomy 29.7%, 13.6% danazol, 20.5% micophenolate mofetil and 4.5% azathioprine. Herbs/supplements were used by 47 patients (42.3%). Treatment side effects were reported by 85 (96%) patients on corticosteroids and 5 (25.7%) patients on IVig. At the time of the survey 66.7% patients had received at least one therapeutic intervention (26 women and 9 men) with a mean age: 32.4 ± 7.1 years (range: 18-48 years) and an equal number of euthyroid healthy subjects (26 women and 9 men) with a mean age: 32.4 ± 7.1 years (range: 20-46 years). Healthy subjects were matched on gender, age (±5 years) and year/month of diagnosis (±1 month). None of the subjects (patients and controls) presented any infectious and neoplastic conditions, diabetes mellitus, hypertension and dyslipidemia. To assess thrombopoiesis, we have determined platelet indices using Sysmex 9000 analyser. Thrombostimulating hormone, thrombomodulin (T3), free-T3, thyroxine (T4) and free-T4 were determined using an electro-chemiluminescence immunoassay intended for use on Elecsys 2010 analyser (Roche Diagnostics, Indianapolis, USA). Statistical analysis of data was performed with SPSS® version 17 for Windows software. Results. Cases presented significantly higher MPV (mean ± SD: 12.27 fl ± 0.46) and PDW (mean ± SD: 15.3 ± 1) than controls (mean MPV ± SD: 10.65 fl ± 0.78, p<0.001 and mean PDW ± SD: 13.5 ± 1.34, p<0.001). On the contrary, patients with subclinical hyperthyroidism had similar number of platelets per mm3 than healthy euthyroid subjects (mean PLT in patients: 253 x 109/mm3 ± 34 versus mean PLT in controls 264 x 109/mm3 ± 38, p=0.15). In a linear regression model, adjusting for age, gender, body mass index and smoking status, the presence of subclinical hyperthyroidism was the most significant predictor of MPV and PDW levels (p<0.001). Conclusion. These results suggest that subjects with subclinical hyperthyroidism tend to present increased platelet size and activation. Elevated platelet activation could contribute to an increased risk of atherothrombotic complications observed in SCH. Finally, these findings suggest that platelet morphologic changes observed in subclinical hyperthyroidism, such as higher MPV and PDW could be attributed to metabolic parameters.
Table 1.

<table>
<thead>
<tr>
<th>ITP-PAQ Scale Scores by Age and Gender</th>
<th>Baseline</th>
<th>Week 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value for change</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall Quality of Life (OQL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity</td>
<td>0.4</td>
<td>0.6</td>
</tr>
<tr>
<td>Bother</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>Health</td>
<td>0.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Pain</td>
<td>0.7</td>
<td>0.5</td>
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</tbody>
</table>

**Summary/Conclusions.**

Methods for both the subgroups (splenectomized patients and those who were not exposed to romiplostim within 24 weeks) were higher than the change for the all patient cohort. The greatest improvement for splenectomized patients was in Bother (12.2 point-change), Activity (11.1 point-change) and Overall OQL (12.0 point-change). Similarly, change scores for patients who had previously received placebo were also highest in these three scales, Bother (9.2 point-change), Activity (7.9 point-change) and Overall OQL (11.8 point-change), with the addition of Reproductive Health (6.6-point change). It is likely that some of these subgroup differences were confounded by the fact that patients that received romiplostim as part of the earlier study (prior romiplostim patients) entered this current study with higher ITP-PAQ scores, therefore resulting in less room for improvement. Some change scores within the subgroups (e.g., Bother, Overall OQL) were also clinically meaningful since they exceeded the 8-point threshold. Summary/Conclusions. Adult patients with chronic ITP who received romiplostim for 48 weeks had significant improvement in all aspects of HRQOL. Change scores for both the subgroups (splenectomized patients and patients who were not exposed to romiplostim within 24 weeks) were higher than the change scores for all patients and also reflected significant improvements. Additional research is needed to more fully compare baseline ITP-PAQ scores and change scores during the course of the study.

**0793**

**BONE MASS AND BIOCHEMICAL MARKERS OF BONE TURNOVER IN CHILDREN AND ADOLESCENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA: RELATION TO CORTICOSTEROID THERAPY AND VITAMIN D RECEPTOR GENE POLYMORPHISMS**

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Background. Chronic immune thrombocytopenia (ITP) develops in less than 15% of childhood acute ITP. Management is usually conservative, however in compromised patients optional drug therapy includes standard oral steroids, pulsed high dose steroid therapy, IVIG, anti-D, and immunosuppressive therapy or thrombopoietin receptor agonists in refractory patients. Objectives. As steroids play an important role in the management of ITP whether acute or chronic, this work aimed to study the bone mass in children and adolescents with chronic ITP in relation to biochemical markers of bone turnover, cumulative steroid therapy, and the possible modulating effect of vitamin D receptor (VDR)gene polymorphisms. Methods. 36 children and adolescents (mean age 10.67±4.72ys) with chronic ITP were recruited from the Hematology Clinic, Children’s Hospital, Ain Shams University, and the Hematology Clinic of the National Research Centre in Egypt, compared to 43 healthy age and sex matched controls (mean age 9.32±2.99 years). The files of the patients were revised and the total cumulative dose of steroids was calculated. After informed consent, patients and controls were subjected to clinical assessment, CBC, bone markers (serum osteocalcin and propeptide I precollagen (CPIP) and urinary deoxypyridinoline excretion). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to analyze VDR gene distribution (Bsm1 and FokI) in patients and control groups. Bone mass was assessed by dual energy X-ray absorptiometry (DEXA) at lumbar and hip regions to measure the bone mineral density (BMD). Results. The duration of ITP ranged from 2-10 years (mean 4.4±3.9 years). Compared to controls, ITP patients had higher BMD (P=0.02) and lower height for age SDS (P<0.001). Patients had lower levels of osteocalcin (P<0.001) and CPIP (P<0.001) and higher urinary DPD excretion (P<0.001) compared to controls. BMD was significantly lower in chronic ITP compared to controls for both spine and hip z-score. BMD was not significantly correlated to serum osteocalcin and CPIP, however urinary deoxypyridinoline excretion was inversely correlated to BMD (P=0.015 and P=0.006 for spine and hip z-score, respectively). BMD in ITP was inversely correlated to age (P<0.05), BMI (P<0.01), and cumulative steroid dose (P<0.01), but not to the disease duration. The correlation between cumulative steroid dose and BMD was highest in patients receiving frequent oral steroids on daily or alternate days protocols (P=0.014 for spine and P=0.001 for hip z score), while the correlation was non significant in patients receiving monthly pulsed intravenous steroid therapy (P=0.74 for spine and P=0.505 for hip z score). There was no relation between BMD and Bsm1 polymorphism, however FokI polymorphism was significant for spine (P=0.038 for spine, P=0.024 for hip z score). Conclusion. High cumulative doses of corticosteroids increase bone resorption in young chronic ITP patients. FokI gene polymorphism could be an accentuating factor in steroid induced bone resorption. Monthly pulse high dose steroids has less effect on bone mass than daily or alternate days oral steroid therapy. Protocols of therapy of ITP should restrict chronic steroid use in growing children and favour alternative less harmful therapies.

**0794**

**SINGLE NUCLEOTIDE POLYMORPHISMS OF THE INFLAMMATORY CYTOKINE GENES IN ADULT PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA**

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Background. Immune thrombocytopenic purpura (ITP) is an autoimmune disease characterized by thrombocytopenia due to platelet autoantibodies, causing an accelerated clearance of opsonized platelets by the reticuloendothelial system. The etiology of ITP remains unclear and genetic and environmental factors are thought to play role in the development of the disease. Aim. The aim of our study was to investigate a possible association of some single nucleotide polymorphisms (SNP) in genes for interleukin beta IL-1β(-511 C/T), tumor necrosis factor beta TNF-β(-308 G/A) and tumor necrosis factor alpha TNF-α(-308 G/A) with ITP. Methods. We have analyzed 125 unrelated adult patients with ITP (35 men and 90 women) with median age of 47 (range 14-83) and 120 healthy matched controls. The median follow up of the patients was 44 months (12-84). Refractory ITP was defined as platelet count lower than 50x109/L despite treatment with standard dose of corticosteroids and splenectomy. All 125 patients were initially treated with corticosteroids, 38 of which were splenectomized. Forty two (34%) patients were refractory to corticosteroids and splenectomy and were defined as having refractory ITP. Genotyping was performed by using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. The distribution of genotypes and allele frequencies were compared between patients and controls using a chi-squared test or Fisher’s exact test. Results. Our results demonstrated significantly different distribution of the TNF-β (+252 G/A) and tumor necrosis factor alpha TNF-α (+308 G/A) genotypes in patients with ITP (n=125; G/G=32, A/G=38, A/A=55) compared with controls (n=120; G/G=30, G/A=36, A/A=54). Allele frequencies for TNF-β (+252 G/A) were also significantly different in patients with ITP (A allele 82.4%, G allele 17.6%) compared with controls (A allele 72.1%, G allele 27.9%), p=0.009 with Yates correction. We didn’t found significant differences in the genotype distribution or al-

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lele frequencies for two other genes. Allele frequencies for TNF-α (-308 G/A) were 8.4% for A allele and 91.6% for G allele in patients and 11.3% for A allele and 88.7% for G allele in controls, p=0.363 with Yates correction. For IL-β (-511 C/T) allele frequencies were 69.2% for C allele and 30.8% for T allele in patients and 70.4% for C allele and 29.6% for T allele in controls, p=0.845 with Yates correction. We found significantly different genotype distribution of TNF-α (-308 G/A) between patients with nonrefractory ITP (n=88; G/G=75, A/G=8, A/A=0) and refractory ITP (n=42; G/G=30, A/G=11, A/A=1), p=0.016. Allele frequencies for TNF-α (-308 G/A) were also significantly different in patients with refractory and nonrefractory ITP (A allele 15.5% versus 4.5%), p=0.009 with Yates correction. There was no significant difference in genotype distribution and allele frequencies for TNF-α (-252 G/A) and IL-β (-511 C/T) between these two groups of patients.

Conclusion. The obtained data indicate that the A allele of TNF-α (+252) is more frequent in patients than in controls and that this polymorphism may play role in disease susceptibility. The A allele of TNF-α was significantly more frequent in patients with refractory ITP, indicating that this gene polymorphism may contribute to therapy resistance and refractory form of ITP.

0795
INCIDENCE OF ANTIPHOSPHOLIPID ANTIBODIES AND THEIR SIGNIFICANCE IN THE PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA
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Background. The presence of antiphospholipid antibodies (APA) in immune thrombocytopenic purpura (ITP) has been reported, but their pathogenic role and clinical importance is not clear. Aims. In this investigation we estimated the incidence of APA’s presence and their influence on development of antiphospholipid syndrome (APS) and systemic autoimmune diseases (SAID) in patients with ITP. Methods. This study involved 59 adults patients with ITP. They were pretreatment evaluated for platelet count (<50x10⁹/L vs ≥50x10⁹/L), presence of APA: lupus anticoagulant (LA) and anticardiolipin immunoglobulin G/M (IgG/M) antibodies (ACA) and antinuclear antibody (ANA). According age, sex, platelet count and APA positivity, the incidence and risk factors for development of APS (thrombosis or fetal losses) and SAID were evaluated. The follow-up risk factors were identified using the univariate and multivariate analysis. Results. The median age of patient was 32 years, range 18-79 years, 69.3% were female and 30.7% were male. At presentation od disease, the platelet count <50x10⁹/L had 70% of patients and 30% had ≥50x10⁹/L. The presence of APA detected in 48% of patients: ACA and LA alone in 12% and LA alone in 25.2% of patients. At presentation of disease, the incidence of APS was significantly higher in patient with positive APA positivity and platelets <50x10⁹/L (p<0.05). During the follow-up period, the incidence of development APS was significantly higher in group of patients with LA positivity (p=0.001) and ACA IgG class positivity (p=0.001). The incidence of development SAID was significantly higher in female patients (p=0.004), age over 32 years (p=0.027) and in patient with LA positivity (p=0.039) and ANA positivity (p=0.005). The univariate analysis identified male sex (p=0.010) and both ACA and LA positivity (p=0.007) as a significant risk factors for development of APS in patients with ITP. Multivariate analysis proved that most significant risk factor for development of APS was both ACA and LA positivity: p=0.007, relative risk (RR) = 0.063 (95% CI 0.008-0.471). The univariate analysis also identified age over 32 years (p=0.022) and LA positivity (p=0.057) as the significant factors for development of SAID in patients with ITP, while multivariate analysis indicated the LA positivity as the most significant risk factor for development of SAID in patients with ITP: p=0.021, RR=0.047 (95% CI 0.004-0.628). Conclusions. The inverse correlation between APS and platelet count, in presence of APA positivity, was found. The most significant risk factor for development of APS in patients with ITP was positivity of ACA and LA mutual, while most significant risk factor for development of SAID was LA positivity. It may be concluded that estimation of APA at presentation of ITP is important for identification of risk group of patients for development APS and SAID.

0796
PROLON TED RESPONSE TO ELTROMBOPAG IN PATIENTS WITH CHRONIC IMMUNE THROMBOCYTOPENIA
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Background. Although difficult to quantify, spontaneous remission of chronic immune thrombocytopenia (ITP) has been estimated at 0.1% per year. American Society of Hematology guidelines refer to 12 case series with observation over 61 years, demonstrating a spontaneous response rate of approximately 5% of patients after failing to respond to glucocorticoids, splenectomy, and subsequent therapy. Instances of prolonged response have been reported with different therapies; it is not always possible to distinguish a prolonged response from a remission. Treatments for ITP have traditionally addressed the platelet destruction component of the disease; the advent of thrombopoietin receptor agonists has addressed the impaired production of platelets. Eltrombopag is an oral, nonpeptide thrombopoietin receptor agonist that is approved for the treatment of chronic ITP. In 6-week and 6-month placebo-controlled trials in patients with chronic ITP, eltrombopag increased platelet counts, reduced bleeding, and reduced the need for concomitant ITP therapy. Long-term treatment with eltrombopag is being evaluated in EXTEND, an ongoing open-label extension study in chronic ITP patients who completed a previous eltrombopag study. Aim. To evaluate prolonged platelet responses to eltrombopag in the EXTEND study. Methods. Patients in EXTEND had received eltrombopag for at least one of the following reasons: two 6-week phase 2 and phase 3 studies (TRA100773A/B), a 6-month phase 3 study (RAISE), or a phase 3 study of intermittent treatment (REPEAT). Dosing in EXTEND is individualized according to platelet counts with a goal of maintaining platelets ≥50,000/µL and <200,000/µL while minimizing the use of concomitant ITP medications. Patients remain in the study until withdrawal or commercial availability of eltrombopag. An ad-hoc analysis was conducted to evaluate the proportion of patients in EXTEND who experienced a prolonged response, defined as a platelet count ≥50,000/µL that was sustained for ≥12 weeks after the last dose of eltrombopag, without any rescue therapies. Results. Among 299 patients enrolled in EXTEND between June 2006 and February 2010, a prolonged response was obtained in 14 patients (5%), which is equivalent to 1.4% per year of observation in EXTEND. The median age of these patients was 55 years (range, 20-71 years), and 11 (79%) were female. Five patients had been splenectomized prior to enrollment in EXTEND, and all patients had received at least 1 pharmacologic agent (range, 1-7 prior therapies) before study entry. A median of 26 months (range, 9-128 months) had elapsed since the diagnosis of ITP, and patients had been treated with eltrombopag for a median of 160 days while in EXTEND (range, 14-1107 days). Conclusion. Treatment with eltrombopag in the EXTEND study was associated with a prolonged response in 5% of patients over 4 years, which is higher than the expected proportion of spontaneous remission. However, it is not possible to assume that the patients with a prolonged response also experienced remission of chronic ITP, because subsequent follow-up is not available for most of these patients.

References

0797
SPLENECTOMY IN CHILDREN WITH CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA - RETROSPECTIVE STUDY-SINGLE CENTRE EXPERIENCE
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Background. The management of chronic idiopathic thrombocytopenic purpura (ITP) and the decision to perform the splenectomy in children with chronic ITP remain two controversial issues. Aims. The aim of this retrospective study was to analyse our centre experience with splenectomy in children with chronic idiopathic thrombocy-
topenic purpura. Methods: We retrospectively examined the records of 25 children who underwent splenectomy for chronic ITP between 1999 and 2009. We studied the time between diagnosis and splenectomy, long term hematological response, morbidity, mortality and predictors of response to splenectomy. Platelet response are categorized as complete response (CR≥150,000/µl), partial response (PR ≥50,000/µl but < 150,000/µl) or nonresponse (NR <50,000/µl). Results: The median age at moment of the splenectomy was 10.6 (3.5-18.2) years. All patients were treated only with intravenous immunoglobulin. Of the high costs of intravenous immunoglobulin. Five patients had a corticosteroid resistant response to splenectomy with recurrent hemorrhagic events and 20 children had a corticosteroid dependent ITP. The median time between diagnosis and the moment of the splenectomy was 12(4-48) months. Presplenectomy vaccination was administered in all patients. After splenectomy was not used antibiotic prophylaxis. The peri-operative mortality was zero and the overall morbidity was 2.5%. The median post-splenectomy follow-up time was 41(4-96) months. The overall immediate response rate was 92% (22 patients had CR and 1 patient had PR). Two children had NR. An improvement in quality of life was observed in 96% of children. During follow-up 2 children relapsed (at 8 and 12 months respectively after splenectomy) and required intermittently corticotherapy. No correlation existed between CR to splenectomy and age, platelet count at diagnosis and last platelet count. All 5 patients with corticosteroid resistant ITP had CR to splenectomy. One of the two children who had NR at splenectomy obtained a stable PR with Rituximab 100 mg/m2weekly iv x 4 weeks. Conclusions: This study shows that splenectomy is effective, provide long-term control of disease and is associated with low morbidity, important improvement in quality of life and a good cost efficiency. Our centre the splenectomy represents an important option for the treatment of chronic ITP in children.

0798
THROMBOPOIETIN RECEPTOR AGONISTS (TPOA) DO NOT CAUSE ACTIVATION OF THE COAGULATION-FIBRINOLYTIC SYSTEM IN PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP)
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Background. TPOa stimulates thrombopoietin receptors, increasing platelet counts (PC) in 70-80% of ITP patients. Venous thromboembolism (VTE) was reported in up to 4% of TPOa trials, triggering speculation regarding TPOa activation of the coagulation process, particularly in the absence of evidence for platelet activation. Aims. This study used D-dimer (DD) as a marker of coagulation and fibrinolysis to determine the effect of TPOa on this system. Methods. We retrospectively evaluated DD in ITP patients on TPOa, performed during routine visits. Two DD assays were used: DD-Dade (cut-off 1.2 mg/L) and DD-HS (cut-off 230 ng/mL). DD-Dade was used in 11/108 analyses and these values were not included in estimating median DD. Results. Median age of 46 patients was 55 years; 40% males. No patient developed VTE during TPOa treatment, in this study. Median time from pre-treatment DD measurement to TPOa initiation was 3 weeks. Median (IQR) pre-TPOa DD (n=40) was 1.3 mg/L (0.7-2.2); PC (n=40) 57 x10^9/L (31-165); F (n=12) 315 (199-345) mg/dL; CRP (n=6) 0.47 mg/dL (0.2-3.7). Median duration from TPOa initiation to first available DD on treatment was 12 weeks. Median (IQR) DD on treatment (n=38) was 1mg/L (0.5-2); PC (n=38) 57 x10^9/L (51-165); F (n=7) 519 mg/dL (203-867); CRP (n=8) 0.4mg/dL (0.1-0.7). No significant difference was found between pre- and on-treatment DD values (p=0.1) although DD tended to decrease with the duration of TPOa treatment (Figure). DD for both assays were positive (n=24) vs negative (n=16) DD pre-TPOa initiation was: 42% vs 25% IVIG; 38% vs 19% steroid; 13% (but 8% were also on IVIG) vs 6% rituximab; 0 vs 19% Rigel; and 17% vs 44% no treatment (p=0.05). There was no correlation between paired PC and DD values pre-treatment, 4, 8 or 12 months during treatment. Conclusions. This study does not support the evidence for increased VTE in ITP patients treated with TPOa being related to a general activation of the coagulation system. Treatment of ITP with TPOa did not activate the coagulation-fibrinolytic pathway as evidenced by no increase in DD following initiation of TPOa. In fact, DD tended to decrease while patients were receiving ongoing TPOa treatment. There is a suggestion, although not a significant difference, that the pre-treatment DD may be related to the therapy, or lack of, received at that time. Further studies are needed to determine the pathoetiology of the infrequent TPOa-associated-VTE.

0799
XENOTROPIC MURINE LEUKAEMIA-RELATED VIRUS (XMRV) AND IMMUNE THROMBOCYTOPENIA (ITP)
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Background. Fatigue in ITP results in decreased health-related quality of life (HRQoL); its pathogenesis is unknown. Emerging evidence suggests that T regulatory cells, natural killer cells, innate immune dysfunction and cytokine imbalance, in addition to organic causes such as hypothyroidism or iron deficiency, may lead to fatigue in ITP. Xenotropic murine leukaemia-related virus (XMRV), a human gammaretrovirus, has been associated with chronic activation of the innate immune system and natural killer cell dysfunction in chronic fatigue syndrome (CFS). XMRV is present in 2-4% of the general population. Aims. The aims of this study were to assess the level of fatigue expressed and to determine any associations between fatigue and XMRV in patients with ITP compared to healthy controls. Methods. Blood samples were collected prospectively with consent at routine clinical visits from ITP patients and from healthy controls, older than 15y, for complete blood count and blinded XMRV detection via: serological techniques to detect XMRV/MRV Env antibody protein; RT-PCR detecting viral RNA; and isolation in culture. Fatigue was tested concurrently for every subject, using the standardized Multidimensional Fatigue Inventory (MFI) questionnaire: a high score reflects severe fatigue. Results. 36 patients with ITP, mean age 50y (range 19-84y), 50% female. Eight of 20 (40%) patients with ITP were positive for XMRV compared to 3/13 (23%) controls (p=0.419). Mean MFI score for every scale was worse for ITP patients than controls (Table). There was no difference in fatigue on any scale between ITP patients XMRV positive and negative. Three patients were on immune suppression, of whom 44% no treatment (p>0.05). There was no correlation between paired PC and DD values pre-treatment, 4, 8 or 12 months during treatment.

Table 1. MFI scores, mean (+/- SE).

Figure 1.
2 (67%) were positive for XMRV, compared to 3/10 (30%) with a splenectomy; 6/12 (50%) on thrombopoietin receptor agonists (TPO-A); 1/3 (33%) on IVIG; none of 2 on GMA161 (an anti-FcγRIII antibody); and none of 3 on no treatment. Among those patients testing positive for XMRV, the mean platelet count was not significantly lower. The platelet count, median 207 x10^9/L (range 8-1160), was associated with fatigue for Reduced motivation (p=0.022) and showed trends for General fatigue (p=0.081) and Physical fatigue (p=0.105). Increasing disease duration, median 17 years (range 3.25-32), tended to have worse scores for Mental fatigue (p=0.128). Summary/Conclusions. ITP patients in this study population expressed higher levels of fatigue than controls across all MFI scales, with an association between lower platelet counts and worse scores for 2/3 scales, illustrating again the importance of fatigue as a manifestation of ITP. This study demonstrates a 40% incidence of XMRV in patients with ITP and 23% in healthy controls. There was no difference in fatigue expression between patients who were XMRV positive and negative, thus XMRV may not be involved in the pathogenesis of fatigue in ITP. Given the small numbers and preliminary nature of the results there may be a potential association between immune suppression and XMRV infectivity. Studying more patients may clarify clinical associations of XMRV in ITP.

**Table:**

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<th>Scale</th>
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<tr>
<td>Mental Fatigue</td>
<td>184</td>
<td>175</td>
<td>172</td>
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<tr>
<td>Reduced Motivation</td>
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<td>175</td>
<td>172</td>
</tr>
<tr>
<td>Emotional Fatigue</td>
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<td>General Fatigue</td>
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*Background.* The mortality of Invasive Aspergillosis (IA) still affects from 27% to 55% of high risk hematologic patients. The reasons of such a poor outcome also rely on several drawbacks limiting the diagnostic accuracy of non cultural based diagnostic methods (NCBDM) and hampering the opportunities for an early intervention. Studies in mice model of IA and in healthy subjects have shown that Aspergillus-specific T-cells producing interferon-gamma (IFN-gamma-T1) are protective, while Aspergillus-specific T-cells producing interleukin-10 (IL-10-T2) are non-protective. We have investigated whether the identification of Aspergillus-specific IFN-gamma-T1 and/or IL-10-T2 through an ex-vivo enzyme linked immunospot (ELISPOT) assay may be effective in the diagnosis of IA in high risk patients. Furthermore, in the proven IA patients, we have functionally and phenotypically characterized such T cells through the cytokine secretion assay (CSA). Methods. 180 patients (168 hematologic and 12 solid organ transplant patients) have been enrolled. They were classified, according the revised EORTC/MSG criteria, as follows: 18 proven, 55 probable, 17 possible IA cases and 110 controls. The control patients were divided in two groups: group 1 included 86 (78.2%) patients with histological and/or cultural verified infectious/inflammatory/neoplastic diseases, but other than IA; group 2 included 24 (21.8%) patients without clinical and/or microbiological features of IA. ELISPOT has been performed, as described [Potenza et al. Leukemia 2007; 21: 578-81], by using as antigens Aspergillus either conidia or recombiant antigens, namely CRF1p, GEL1p, PEP1p, SOD1p, α1-3 glucan, β1-3 glucan and galactomannan (GM). Results. The patient and sample positivity rates were 94.4%/89.5% in proven, 45.7%/35.3% in probable, 35.3%/50% in possible IA cases and 1.8%/4.5% in the controls, respectively. The sensitivity and specificity of ELISPOT for the diagnosis of IA resulted 94.4% (95% CI, 75%-99%) and 98.2% (95% CI, 93%-99%), respectively. The PPV of the test was 89.5% (95% CI, 67%-99%), the NPV was 99.1% (95% CI, 94%-100%) and the efficiency was 97.6% (95% CI, 92.3%-99.4%). The positive likelihood ratio (LR) resulted 51.89, the negative LR was 0.06 (Table 1A,B). In proven IA patients, CSA demonstrated that Aspergillus-specific IL-10-T2 were predominantly central memory (CM) CD4+ T cells (median frequency 0.37%/0.22%), while Aspergillus-specific IFN-gamma-T1 were either CD4+ or CD8+ cells of either effector memory (EM) or CM phenotype (median frequencies 0.24%/0.20%). Also lower frequencies of Aspergillus-specific either CD4+ or CD8+ T cells producing IL-4 (0.11%/0.19%) of EM phenotype, and EM CD8+ cells producing IL-17 (0.18%), were detected. Moreover, although CRF1p, GEL1p, α1-3 glucan and SOD1p resulted the antigens eliciting the highest number of Aspergillus-specific IFN-gamma-T1, only GEL1p and α1-3 glucan were those most constantly targeted by protective immune responses along the entire course of the IA. Conclusions. Our findings demonstrate the potential of ELISPOT in the diagnosis of IA, suggesting that it may complement the other NCBDM, enabling a more consistent diagnosis of IA. Furthermore, this study describes for the first time the Aspergillus-specific immune responses in patients with proven IA, identifying...
also the antigens predominantly targeted by protective IFN-gamma-T1, with possible consequences in designing strategies of either adoptive cell infusion or vaccine therapies.

**0801**

**SURVEY ON ANTIFUNGAL COMBINATION THERAPY FOR TREATMENT OF PROVEN OR PROBABLE INVASIVE FUNGAL DISEASES IN ITALIAN HEMATOLOGICAL CENTERS. THE SEIFEM-COMBO STUDY**


1University of Udine, Udine, Italy 2Department of Hematology, Università Cattolica Sacro Cuore, Rome, Italy 3AHEMATOLOGICAL CENTERS. THE SEIFEM-COMBO STUDY OF PROVEN OR PROBABLE INVASIVE FUNGAL DISEASES IN ITALIAN SURVEY ON ANTIFUNGAL COMBINATION THERAPY FOR TREATMENT

Table 1. A.B. Diagnostic Accuracy of the ELISpot assay for the diagnosis of Invasive Aspergillosis (IA).

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A.B. Diagnostic Accuracy of the ELISpot assay for the diagnosis of Invasive Aspergillosis (IA). The results of this study indicate that: 1) Combo was well tolerated in both children and adults hematologic pts. 2) The Overall Response Rate was 73% and the mortality IFDs related was only 17%. 3) The most used Combo regimens were Caspofungin+Voriconazole (ORR 80%) and Caspofungin+L-AmB (ORR 70%). 4) In univariate and multivariate analysis PMN recovery during Combo predicts a better outcome.
**Methods.** The 2009 H1N1 vaccine in patients with hematologic malignancies.

**Aims.** A novel vaccine against the virus strain A/California/07/2009 (H1N1) was developed to protect healthy volunteers, while its efficacy in immunocompromised patients, in particular those with cancer, is unknown. Aims: The aim of this study was to determine the safety and efficacy of the 2009 H1N1 vaccine in patients with hematologic malignancies.

**Methods.** We prospectively evaluated the humoral and cellular immune responses after one injection of monovalent adjuvated influenza A/Caliifornia/2009(H1N1)-like strain surface antigen vaccine in 47 adults with hematologic malignancies and 77 adult controls. Antibody titers were measured by hemagglutination-inhibition assay and virus-specific T-cell responses by flow-cytometry on days 0, 28, 50 and 90 after injection. Results. Of the 47 patients, 15 had lymphoma, 21 multiple myeloma, 9 myelodysplastic syndrome or leukemia. Patients were divided into two treatment regimens: 14 received the vaccine, 13 were recipients of allogeneic hematopoietic stem cell transplant (HSCT). By day 28, immunologic response was lower in the patient cohort relative to controls by all parameters (p<0.05). At subsequent time points, seroprotection (antibody titters ≥ 1:40) rates and geometric mean titters (GMT) increased in the patient group and were not significant different relative to healthy volunteers. In marked contrast, seroconversion rates (≥ 4 fold increase in antibody titter) were lower in the patient group also at later time points (p<0.05). Subgroups analyses were performed to evaluate the influence on vaccine efficacy of follow-up, treatment, previous HSCT. Patients in follow-up had immunologic responses similar to controls at all time points. Conversely, patients receiving treatment had lower seroprotection rate on day 28 and lower seroconversion rates on day 28 and day 50 relative to controls. Of note, within the treated group, patients receiving immunomodulatory drugs (IMIDs) displayed a trend for improved seroprotection (day 90, 100% vs 60%, p=0.06) and increased GMT (day 28, 246 vs 87, p=0.5) compared to other treatments. Patients vaccinated after HSCT had the lowest seroconversion and seroprotection rates on day 28 and day 50 (p<0.01 relative to controls), with a slight increase on day 90. Accordingly, GMT was lower than in controls on day 28 (p<0.001) and subsequently it slightly increased. We also assed the cellular response to H1N1 vaccine by enumeration of fold increase of virus specific CD4+ and CD8+ T-cells on day 21, day 50 and day 90 relative to day 0. In the control group, specific CD4+ cells (6.0 ± 2.4, day 50, p<0.05) and CD8+ cells (21.7 ± 12.0, day 50, p=0.08) significantly increased on day 21 and day 50 relative to day 0. A similar trend was observed in the patient cohort for specific CD4+ (28.0 ± 11.0, day 50, p<0.01) and specific CD8+ cells (16.5 ± 5.8, day 50, p<0.05). Summary Conclusions. Patients in follow-up or receiving IMIDs were protected by H1N1 vaccination. In contrast, patients receiving other types of treatment and those treated with HSCT responded poorly. These results may contribute to improve the vaccination strategy especially for poor responders.

**Table 1.**

**0804**

**PROPHYLAXIS OF INVASIVE FUNGAL DISEASES WITH POSaconazole IN ACUTE MYELOID LEUKEMIA. A REAL LIFE EXPERIENCE**

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**Background.** Acute Myeloid Leukemia (AML) patients are at high risk of Invasive Fungal Diseases (IFDs). We report our real-life experience with POS prophylaxis in AML. We also compare the performance of POS prophylaxis with an historical, well matched, control group of AML pts who received prophylaxis with Fluconazole (FLUCO) or Itraconazole (ITRA).

**Patients and Results.** Fifty-five unselected and consecu- tive AML pts received POS prophylaxis (600 mg daily) between Jan 2009 and Oct 2010. Median age of this population was 47 yrs (range 18-69). All cases were given chemotherapy with anthracyclines and cytarabine. The POS was started when neutrophil (PMN) count was less than 1000 mL and was stopped at PMN recovery. The median duration of severe neutropenia (PMN below 500 mL) was 15 days (range 7-41). 10/55(18%) of cases had an oral mucositis grade II-III CTC (common toxicity criteria) and 73%(4/55) of these pts received a proton pump inhibitor. An active diagnostic work up was made in all cases with Galactomannan assay, standard chest X-ray and thoracic CT scan in case of fever (FUO) lasting over 48 hours. The median duration of POS prophylaxis was 15 days (range 7 to 41). Only 4/55(7%) of pts required parenteral empiric or pre-emptive antymycotic therapy and only 2/55(4%) experienced a proven IFDs (Fusarium solani fungemia and Aspergillus sp pneumonia). Mortality IFDs related was 0%. POS was well tolerated and only 9%/55(5) of pts experienced mild drug related side effects. No cases of POS discontinuation, due to the side effects or intolerance, were reported. When we compare the 55 pts who received POS with an historical control group of 55 AML pts who received FLUCO (45/55) or ITRA (10/55) prophylaxis, between Jan 2008 and Jun 2009, no significant differences were observed for underlying disease status, age, IFDs risk factors, days of severe neutropenia and days of prophylaxis.Instead, there were significant differences in breakthrough IFDs (4% in POS group vs 16% in control group; P=0.02), and in days of parenteral antymycotic therapy (57 vs 165). Conclusions. This real-life experience confirms that POS prophylaxis is feasible, safe, well tolerated and effective (prevention of IFDs) in unselected AML patients. Only 7% of these high risk pts required parenteral antmycotic therapy and only 4% experienced breakthrough IFDs. We also confirm that POS is more effective than FLUCO or ITRA as antifungal prophylaxis in AML pts.

**0805**

**ELEVATED COMBINED SERUM FREE LIGHT CHAIN (CFLC) LEVELS ARE SIGNIFICANTLY ASSOCIATED WITH INCREASED MORTALITY**

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**Background.** Though abnormal serum free light chain (FLC) ratios are diagnostically important in almost all plasma cell disorders an absolute rise of polyclonal FLC levels are often discarded as inconsequential. Here we report a striking association between increased combined FLC (summated FLCκ and FLCλ) concentrations and mortality. Aims. To investigate the prognostic impact of elevated CFLC levels and other biomarkers in a haematological referral cohort. Methods. 753 serum samples were sent for various haematological investigations. Serum cFLC, albumin, C-reactive protein (Crp), erythrocyte sedimentation rate (ESR), estimated glomerular filtration rate (eGFR determined by the MDRD equation) and total IgG, IgA and IgM immunoglobulins were measured by standard techniques. Patients with abnormal serum protein electrophoresis, and / or abnormal FLC ratio with confirmed positive IFE were removed. The remaining 528 patients with absolute rise of polyclonal FLC levels were followed for up to 4.3 years. Statistical analysis was performed using SPSS (version 19). Results. Over the 4.3 years of follow-up there were 99 deaths (18.8% of mortality). A Kaplan-Meier survival curve revealed that a large proportion of the deaths were within the first 100 days (29 deaths; 29% of all deaths). As a consequence, Cox regression analysis was performed separately to deter-
mine risk factors for all deaths and deaths within 100 days. The relative risk of death increased proportionally with increasing combined HLC (cHLC) concentrations. Patients with cHLC concentration >50mg/L had a significantly increased risk of death compared to patients with cHLC concentration <50mg/L (75% vs overall survival (OS) <50mg/L, not reached, >50mg/L BSI patients, p = 8.7x10^{-10}). Receiver Operator Characteristic Curves (ROC) analysis indicated that cHLC concentrations >65mg/L was the optimum cut-off associated with mortality in this population (75% vs OS <65mg/L, not reached, >65mg/L, 298 days, p = 3.0x10^{-11} (Figure 1)). Univariate analysis identified albumin <33g/L, Crp>10mg/L, ESR >30mm/1.73m², age >75 and cHLC >65mg/L as being significant predictors of mortality for the whole population and for mortality within 100 days. Gender was associated with mortality within 100 days only. Using multivariate analysis only cHLC concentrations >65mg/L, albumin concentrations <33g/L and eGFR <30mL/min/1.73m² were independently associated with mortality within 100 days and for the entire duration of follow up. Age >75 years old was independently associated with mortality over the duration of follow up but not for mortality within 100 days. A simple risk stratification model based on albumin <33g/L and cHLC >65mg/L identified 86% mortality within 100 days and 62% over the duration of follow up, compared to 50% and 24% using albumin <33g/L and 73% and 50% using cHLC >65mg/L independently. Conclusion. Polyclonally raised cHLC measurements predict significantly high mortality both within 100 days and beyond. cHLC levels can be used in a simple risk stratification model to identify ‘high risk’ patients with increased mortality even in those without B cell disorders.

**0806**

**EMPIRIC ANTIBIOTIC STRATEGIES IN FEBRILE HAEMATOLOGICAL PATIENTS: IMPACT ON EPIDEMIOLOGY AND LETHALITY OF BLOODSTREAM INFECTIONS**

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**Background.** Adequate empiric antibiotic therapy in febrile neutropenic patients is mandatory for a favourable outcome of infectious complications. Epidemiological surveillance of infections occurring at a Haematology Unit is essential to drive an effective antibiotic strategy. Aims. To determine epidemiology, prognostic factors and emerging antibiotic resistance of bloodstream infections (BSI) according to empiric antibiotic strategy adopted. Methods. All BSI consecutively occurred at our Institution during a 78 month period (June 2004-December 2010) were evaluated and correlated with type and state of underlying disease, neutropenia, associated pneumonia, previous antibiotic therapy, including prophylaxis with fluoroquinolones, resistance to antibiotics and outcome. Empiric antibiotic therapy was different during time according to epidemiological data collected. Results. During the entire period of observation, 502 BSI were recorded. Fungi were responsible in 8 (1.6%) cases; Gram-positive (G+) bacteria in 167 (33.3%), Gram-negative (G-) bacteria in 292 (58.4%). In 35 (7%) cases a polymicrobial (PM) BSI was observed. Globally, no significant differences in the distribution of fungal, G+, G- and PM BSI were recorded over time. Empiric antibiotic strategies adopted were ceftriaxone-amikacin from June ‘04 to March ‘06 (21 months: period A), piperacillin/tazobactam-amikacin from April ‘06 to June ‘09 (33 months: period B), ceftazidime-amikacin from July ‘09 to December ‘10 (19 months: period C). BSI/month ratio was 5.7, 6.8 and 6.3 during period A, B and C respectively. P. aeruginosa BSI were more frequent during B and C in comparison with A (21% vs 19% respectively, p=0.005 and 0.04); multiresistant Pseudomonas (MR Psed) were absent in A and accounted for 12% and 9% in B and C respectively. Vancomycin-resistant enterococci (VRE) accounted for 25% and 22% of all enterococcal BSI in A and B, respectively, whereas they were absent in C. Frequency of extended-spectrum β-lactamases (ESBL+) enterobacteriaceae was lower during B (10%) in comparison with A+C (21%) (p=0.057). Overall mortality was 9.6%; it was significantly higher during B in comparison with A+C (12.5% vs 6.2%, p=0.021). P. aeruginosa BSI lethality was absent during A; during B it was higher than during C (31.5% vs 17.4%), although not significantly. MR Psed BSI lethality was significantly higher during B in comparison with C (46.9% vs 9.1%, p=0.038). Among other MR pathogens, 5/7 patients with a VRE BSI died. Univariate analysis showed that un-controlled haematological disease (p<0.0001), P. aeruginosa BSI (p<0.0001) and associated pneumonia (p=0.0014) were statistically significant risk factors for death. Piperacillin/tazobactam-amikacin as empiric antibiotic approach was also associated to increased risk of death at our Haematology Unit (p=0.021). Summary/Conclusions. The empirical approach with ceftazidime-amikacin showed a trend in terms of reduced MR Psed BSI lethality; for this reason at present it is the association still adopted at our Haematology Unit. When piperacillin/tazobactam-amikacin was chosen as empirical antibiotic therapy the frequency of ESBL+ enterobacteriaceae was reduced; however, the impact of ESBL+ BSI on mortality seems to be limited. Further epidemiological surveillance is warranted to monitor emerging resistant strains (particularly ESBL+) and related mortality.

**0807**

**RISKS OF POST-SPLENECTOMY SEPSIS: ARE PATIENTS AWARE? A SURVEY**

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**Introduction.** Splenectomy is associated with a lifelong increase in the risk of sepsis. The aim of this survey was to assess the level of knowledge of patients who have undergone splenectomy in Northern Lincolnshire and Goole NHS Trust. Methods. Adults undergoing splenectomy between 1993 - 2009 in our trust were invited to participate in the survey. Standardised postal questionnaires were completed by them. Results. 245 splenectomies were carried out in that duration. 85 responses were obtained from 145 patients who were alive as per our records in Jan. 2010. Most of them (94%) were aware of increased risks of certain infections. Majority of them (69.6%) didn't think they (or their family) received any counselling before/after the operation. 54% had standby anti-infective medication and 44% kept a note of their splenectomy. Majority of them (69.6%) didn't think they (or their family) had a good knowledge of infection risks of post-splenectomy sepsis, deficiences were identified. We propose the development of a splenectomy protocol and patient briefing to improve patient education to reduce the risks of post-splenectomy sepsis. We also propose the development of a national database for Asplenic/Hyposplenic patients.

**0808**

**FIRST LINE TREATMENT OF PROBABLE AND PROVEN INVASIVE ASPERGILLOSIS WITH CASPOFUNGIN IN ONCOHEMEPUTIC PATIENTS. A SINGLE CENTRE EXPERIENCE**

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**Background.** Infections are the main complication for oncohematopatitc patients (pts) with severe neutropenia. Caspofungin (Caspo) inhibits the growth of the fungal cell wall. Aims. To evaluate the tolerability and efficacy of Caspo. Methods. Between 2004 and 2009 we have treated 70 consecutive adult neutropenic pts with probable or proven invasive aspergillosis with Caspo as first line therapy. In case of persistent fever (5 days) despite broad-spectrum antibiotic therapy, a high-resolution CT-scan of the lungs and galactomannan test were performed. According to the revised EORTC criteria and Cornely et al (CID 2007) we defined as probable the infections with clinical criteria and a highly suggestive CT-
scan. The pts were 39 males and 31 females; the mean age was 56 yrs (range 18-77 yrs). The diagnoses were: leukemia 54 (77%), myeloma 2 (3%), lymphoma 14 (20%); the disease’s phases were: new onset 30 (43%), remission 18 (26%), relapse 22 (31%). Thirteen pts received an allogeneic and 5 an autologous transplant; the other pts received an induction or consolidation or rescue chemotherapy course. Results. Infections were proven in 15 cases (21%: 12 aspergillus spp, 2 aspergillus fumigatus, 1 G. capitatum) and probable in 55 cases (79%). The first site of infection was the lung in 69 pts (99%) and paranasal sinuses in 1 patient (1%). CT scan was positive (halo sign or air-crescent sign) in all the pts with a lung localization, while the chest X-ray was positive in 40% of them. BAL was performed in 36 pts. Galactomannan on BAL and serum was positive in 32/36 (92%) and 30/70 cases (45%) respectively. Caspofungin was administered at the dose of 70 mg i.v. on day 1 followed by 50 mg i.v. in 1 hour daily. The mean time of treatment was 17 days (range 13-25 days). Caspofungin was well tolerated and not discontinued for adverse events. No premedication was necessary. The global (partial and complete) response, defined as clinical and radiological, was 60/70 (86%); 10 pts died for fungal infection. The responses were similar for probable and proven infections. No breakthrough fungal infections were found. All surviving patients, upon discharge from the hospital, received oral treatment with Voriconazole or Posaconazole. Among the 60 responsive patients, 30 (50%) died later: 26 for hematologic disease and 4 for sepsis. In 3 cases there was no evidence of the fungal infection. Conclusions. For all the cured pts, there was a concomitant recovery of neutrophils so also in our experience this appears to be crucial for the resolution of the infection. In conclusion the resolution rate of the infections is very high (86%); Caspofungin seems safe, it does not preclude any other treatment, it is well tolerated and the cost is lower than other antifungal treatments.

0809
THE EFFECTIVENESS OF MEGA-DOSE MELPHALONISOLONE AND FRESH FROZEN PLASMA TREATMENTS IN PATIENTS WITH CRIMEAN-CONGO HAEMORRHAGIC FEVER ASSOCIATED WITH REACTIVE HAEMOPHAGOCYTIC LYMPHOHISTIOCTYSIS
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Background. Crimean Congo Hemorrhagic Fever (CCHF) is characterized by a macrophage activating syndrome, which starts during the period when viremia decreases. A cytokine storm initiates and triggers the development of haemophagocytic lymphohistiocytosis (HLH). Cytokines mediating vascular dysfunction causes disseminated intravascular coagulation (DIC). The causes of hemorrhage are DIC, thrombocytopenia and endothelial damage. DIC may develop due to direct damage of endothelial cells by the virus, liver dysfunction and cytokine storm (increase in interleukin (IL)-1, IL-6, IL-12, IL-18, tumor necrosis factor (TNF)-α and interferon (IFN)-γ). Aims. The effectiveness of mega-dose methylprednisolone (MDMP) (5-30 mg/kg/d) and fresh frozen plasma (FFP) (15 ml/kg 1-3 doses in a day) in addition to the MDMP and FFP treatments because the patients had resistant thrombocytopenia. Results. All patients were successfully treated with MDMP and FFP. Fever decreased under 37°C in 1.6 ± 0.9 days, WBC increased above 4500/ul in 4.0 ± 2.1 days, platelets increased above 150.000/ul in 8.6 ± 5.3 days and D-dimer decreased under 37°C in 5.9 ± 3.8 days. Conclusions. We support that CCHF associated with reactive HLH should be treated with MDMP and FFP to supress the macrophage activation and cytokine storm, and to complement the deficient coagulation factors due to disseminated intravascular coagulation, respectively.

0810
CHARACTERISTICS AND RISK FACTORS FOR ABSCESES IN A CONSECUTIVE COHORT OF PATIENTS WITH ACUTE MYELOID LEUKEMIA PATIENTS: A TERTIARY CARE CENTER EXPERIENCE
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Background. Abscesses may occur as an infectious manifestation in acute myeloid leukemia (AML) patients. Early reports primarily fo-
cused on anorectal infection in leukemic patients only. Recently, artic-
les of leukemic patients developing abscess mostly focused on brain abscesses or fungal infections and were case reports only. There were no systematic analyses done before regarding the mortality, prevalence, risk of abscess, survival, or treatment consensus in leukemic patients diagnosed of abscesses. Aims. We focused on the prevalence of abscess in patients with AML and the clinical characteristics, risk factors, and prognosis of AML patients developing abscess. Methods. 354 newly diagnosed patients were retrospectively analyzed. Eligible patients were sub-grouped as abscess group (n = 54) and non-abscess group (n = 300). We determined the factors potentially associated with abscess incidence. Results. The prevalence of all kinds of abs-
cesses in AML patients is 9.6% with predominant sites at perianal and hepatosplenic abscesses. Bacteria were the major pathogen of ab-
scesses. The two independent risk factors predicting abscess develop-
ment in AML patients are secondary AML and receiving intensive in-
duction (P = 0.001; HR = 3.739; P = 0.002; HR = 3.775, respectively). We categorized patients according to the numbers of two inde-
pendent risk factors they possessed (0, 1, and 2 risk factors). Figure 1 showed the cumulative incidence of abscess within 36 months after AML diagnosis according to the risk stratification system (1.7% vs. 12.4% vs. 22.2%, 0 factors vs. 1 factors vs. 2 factors, P = 0.001). Pa-
tients developed abscesses in non-remission status of AML are asso-
ciated with higher post-abscess 100 days mortality (92.3% vs. 42.0%, P = 0.004). Conclusion. Abscesses are not uncommon in AML patients. Patients who have secondary AML and receiving intensive induction chemotherapy warrant special attention since they are prone to have abscess. Leukemic free status at abscess diagnosis predicts a better survival in AML patients with abscess.

0811
MORPHOMETRIC NEUTROPHIL DATA FOR EARLY DIAGNOSIS OF SEPSIS IN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES
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Background. Early diagnosis of serious blood stream infections is es-
cessful to improve treatment and survival of patients with haematolo-
gical diseases. C-reactive protein (CRP) in combination with neutrophil count and the manual band counts in blood smears are often used for predicting systemic clinical infections (sepsis). However CRP rises several hours after the onset of infection, so it can be falsely negative; on the other side, the manual myeloid immaturity differential count is a time consuming procedure, requiring well-trained skills of the labora-
tory technicians. Neutrophils are early activated by mediators of in-
flammation in systemic infections and rapidly change their volume and granularity. Aim: To investigate the clinical usefulness of additional morphometric data from automated hematocline analyser of reactive neutrophils for predicting systemic clinical infections (sepsis) in patients with haematological malignancies. Methods. Morphometric re-
search population data (Volume, V; conductivity, C; Scatter; S) were ob-
tained from 100 patients using the Beckman Coulter Dimension V-w hemato-
lyal G antewiser during automated differential counts of peripheral blood of 134 consecutive adult haematologcal patients with positive blood culture and nucleic acid amplification tests for Gram (+) and

Figure 1. Cumulative incidence of abscess within 36 months.
S Magrin, F Fabbiano, A Malato, A Luppino, R Pipitone, MG Donà, D Turri, R Felice (PICCS) IN HAEMATOLOGICAL PATIENTS

SAFETY AND EFFICACY OF AN EDUCATIONAL PROGRAM IN REDUCING COMPLICATIONS OF PERIPHERALLY INSERTED CENTRAL CATHETERS (PICCS) IN HAEMATOLOGICAL PATIENTS

A Malato, A Luppino, R Pipitone, MG Donà, D Turri, R Felice, S Magrin, F Fabbiano

Purpose. Patients with haematological disorders frequently require the insertion of medium or long-term central venous catheters (CVCs) for stem-cell transplantation, the administration of chemotherapy, or transfusion of blood products. Although peripherally inserted central catheters (PICCs) have been in use for many years, little data exist about their use in patients receiving intensive chemotherapy.

Methods. Evidence-based interventions were implemented in our department in December 2010, and included: 1) An high level nurse education program for correct practices and prevention of catheter-associated complications. was developed for PICC nursing team; 2) The use of ultrasound guide for the insertion of the tip of PICCs, thanks to a special operator training; 3) Bedside placement and confirmed PICC tip placement by chest radiography after removal of the guidewire and before the securing of the catheter; 4) Maintenance of maximum sterile barrier precautions during PICC insertion and aftercare; 5) Chlorhexidine preparation, replace 10% povidone iodine for skin antisepsis; 6) adoption of PICC patient nurse archive, including the information of weekly PICC line review at our department for each patient. Results. Ninety-five PICCs were in place for a total of 7,295 PICC days (range, 1-331 days; mean, 76.7 days). Sixty-six PICCs were inserted during severe thrombocytopenia (platelets < 50 x 10^9/L), and 70 during severe neutropenia (neutrophils < 0.5 x 10^9/L). The majority of the patients were affected by leukaemia, and PICCs were inserted to ensure adequate access throughout chemotherapy. There were 2 thrombotic complications PICC-related (0.27 per 1,000 CVC days), and only one CRBSI (0.15 per 1,000 CVC days) during neutropenia. Other mechanical complications occurred in 11 catheters, and were accidental dislodgement (4), catheter break (5), catheter inadequate (4). Conclusions. Our results indicate that a training and competence assessment program is effective in reducing the main complications PICCs-related in haematological setting.

VORICONAZOLE PLASMA LEVELS AND GENETICS POLYMORPHISM OF CYP2C19 IN HEMATOONCOLOGICAL PATIENTS

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Background. Voriconazole (VORI) is a broad spectrum antifungal which exhibits a wide spectrum of its plasma concentrations. Among others, differences in drug metabolism due to polymorphic gene expression of CYP2C19, is discussed as an possible reason for this variability of VORI plasma concentrations. Aims. To evaluate the genotype of
CYP2C19 in hematopoietic patients treated with VORI in our institution and its correlation with VORI plasma concentrations obtained during the VORI treatment. Methods. Trough VORI plasma concentration were measured using a high performance liquid chromatography assay. A retrospective analysis of laboratory results from patients treated with VORI between August 2005 and January 2010 was performed. The genotype status of CYP2C19 was determined retrospectively using a polymerase chain reaction. Results. From 122 patients included in the study 90 patients have got wild type in both alleles of CYP2C19. 32 patients have got heterozygous mutations. There was no patient with homozygous mutations in our study. In our patient group VORI was administered in 40.7% as prophylaxis, in 13.5% as an empirical antifungal treatment and in 45.8% as a preemptive treatment or treatment of proven of invasive fungal infection. VORI was administered mainly orally; only in 2% of 481 analyzed samples intravenously. The median plasma concentration (after standard daily dose 400 mg orally) was in the wild type group 1.04 µg/ml and 1.73 µg/ml in the group with heterozygous CYP2C19 mutations. The VORI plasma concentration was < 1.0 µg/ml, below the level associated with a better response to treatment of invasive aspergillosis, in 47.7% in wild type group and in 30% in heterozygous group. There was a significant difference between medians of VORI plasma concentration in group with wild type and group with heterozygous mutations - 1.04 and 1.78 (p=0.014, median test). The results are in Table 1. Conclusion. The knowledge of genotype status of CYP2C19 could be an useful guide during the antifungal treatment. Together with VORI plasma concentrations monitoring help us to achieve the plasma and tissue concentrations and improve the outcome of invasive fungal infection in hematopoietic patients.

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0815

CLONAL PATTERNS OF X-CHROMOSOME INACTIVATION IN FEMALE PATIENTS WITH CHRONIC IDIOPATHIC NEUTROPENIA USING REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION METHOD

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Background. Chronic idiopathic neutropenia (CIN) is a disorder of granulopoiesis characterized by increased apoptosis of the granulocytic progenitor cells and presence of activated, oligoclonal T-lymphocytes with myelosuppressive properties in the bone marrow. The underlying T-cell activating stimulus remains obscure. The possibility of the clonal origin of CIN, that might induce the immune system activation, has never been investigated so far. Aim. To probe the hypothesis of the clonal origin of CIN by looking for clonal patterns of X-chromosome inactivation in peripheral blood (PB) cell subsets of female CIN patients. Methods. We have studied the expression of the polymorphism at nucleotide (nt) 1311 of glucose-6-phosphate dehydrogenase (G6PD) and at nt 438 of iduronate-2-sulfatase (IDS) gene by reverse transcription polymerase chain reaction (RT-PCR), in 77 females fulfilling the diagnostic criteria for CIN and 18 female healthy controls. Lymphocytes were purified from PB samples by density gradient centrifugation whereas granulocytes were isolated from the red cell pellet by Dextran precipitation. DNA extracted from total PB cells was screened for heterozygosity at nt 1311 of G6PD and at nt 438 of IDS. RNA was extracted from the isolated cell subpopulations of individuals showing heterozygosity of at least one of the two genes and assessed further by means of RT-PCR. Samples showing the expression of >95% of one allele in granulocytes and/or lymphocytes were classified as a “clonal pattern”.

Results. Overall, 19 CIN patients and 6 healthy individual heterozygous for G6PD and/or IDS were further studied. Of these patients, 10 CIN patients and 3 healthy individuals were heterozygous for the nt 1311 of G6PD whereas 12 CIN patients and 4 healthy individuals were heterozygous for the nt 438 of IDS. Clonality analysis using the G6PD polymorphism showed that 6 of 10 CIN patients (60%) displayed a clonal pattern in both granulocytes and lymphocytes. Two patients (20%) showed a clonal pattern in granulocytes but a polyclonal pattern in lymphocytes. Two patients (20%) showed expression of both alleles in granulocytes and lymphocytes compatible with polyclonality. Clonality analysis using the IDS polymorphism showed that 7 of 12 CIN patients (58.3%) displayed a clonal pattern in both granulocytes and lymphocytes. One patient (8.3%) showed a clonal pattern in granulocytes but a polyclonal pattern in lymphocytes. Four patients (33.3%) showed a polyclonal pattern in granulocytes and lymphocytes. Analysis of samples that were informative for both polymorphisms showed that the results were concordant in every case. None of the controls showed a clonal pattern in G6PD and/or IDS expression in either granulocytes or lymphocytes. Combining the G6PD and IDS results, clonal patterns were observed in granulocytes of 13/19 CIN patients (68.4%) and 0/6 normal individuals (p<0.01).

Summary/Conclusions. Clonal patterns of granulocytes occasionally in association with clonal patterns of lymphocytes are identified in a significant proportion of CIN patients. These data suggest the possible involvement of a prionmutant or myeloid committed clonal stem cell in the pathogenesis of CIN, indicating for first time the possible clonal origin of this disease.

PREGNANCY OUTCOME IN GENETIC SUBTYPES OF CONGENITAL NEUTROPENIA

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Severe congenital neutropenia (CN) comprises a heterogeneous group of disorders with a common hematological and clinical phenotype characterized by a maturation arrest of myelopoiesis at the level of the promyelocyte/myelocyte stage with peripheral blood absolute neutrophil counts (ANC) below 0.5 - 10^9/l and early onset of bacterial infections. Current data on the molecular causes suggest that CN is a multigenic disorder. CN follows an autosomal dominant or autosomal recessive pattern of inheritance. To date more than 10 gene mutations have been described which are involved in disorders associated with CN. Genetic analyses in autosomal dominant and sporadic cases of CN indicate that the majority of these cases are attributable to mutations in the elastase 2 (ELANE) gene encoding neutrophil elastase. However, mutations in the ELANE gene do not discriminate between patients with CN and patients with cyclic neutropenia (CyN), another rare congenital disorder with a cycling haematopoiesis of 21 days. In addition, a number of less frequent other mutations has been identified in recessive CN, which are mainly associated with multi-organ involvement such as p14, SBDS, G6PT, G6PC5, TAZ or WAS. Since 1987, recombinant human Granulocyte-Colony stimulating factor (G-CSF) has been available for treatment of CN. More than 90 percent of patients respond well to G-CSF with a sustained increase of absolute neutrophil counts and a prolonged life expectancy. Since our first patients reach adulthood the desire for parenthood is coming up. In this study we assessed the outcome of 22 pregnancies in 12 mothers and 13 pregnancies from 7 fathers with different genetic subtypes of congenital neutropenias or unknown gene mutation as shown in the table below.

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<th>Miscarriages</th>
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<td></td>
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Total pregnancy outcomes 16 9 7 3
Conclusion. Out of the 32 life births 16 newborns presented with congenital neutropenia documenting the inheritance of the genetic defect from the affected parents. 9 were healthy and in 7 the outcome was not reported. In 3 mothers miscarriage was documented. No other neonatal abnormalities were reported in our cohort, independent of any cytokine treatment during pregnancy. 32 of the 35 other neonatal abnormalities were reported in our cohort, independent of any cytokine treatment during pregnancy. 32 of the 35 other neonatal abnormalities were reported in our cohort, independent of any cytokine treatment during pregnancy. 32 of the 35 other neonatal abnormalities were reported in our cohort, independent of any cytokine treatment during pregnancy.

0817 DOCUMENTED INFECTIONS IN NEUTROPENIA: DATA FROM THE ITALIAN NEUTROPENIA REGISTRY

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Background. Infections are important causes of morbidity/mortality in children with severe congenital neutropenia (SCN), autoimmune (AN) and idiopathic (IN) neutropenia. Few data on the incidence and pattern of infections are available in the literature. Aims. To describe incidence and type of infections in a large population of neutropenic patients. Methods: Patients and Methods. Patients affected with SCN, AN and IN followed from 2000 to 2009 in 8/20 centres of the Italian Neutropenia Registry entered the study. As infections data contained in the INR were not enough detailed for the aims of our study and in order to uniform infection diagnostic criteria, a dedicated CRF was sent to each centre for retrospective data collection. The occurrence of infections was calculated during a period “at risk” defined as person-days at risk divided by the observation time. The infection rate (IR) was calculated by dividing the number of events by the pdr and expressed as episodes/1000 pdr (95% CI). Results. Seventy three patients (28 females and 45 males) of whom 12 (16%) with SCN, 38 (52%) with AN and 23 (32%) with IN were analyzed. At least one infectious episode was observed in 100% of SCN and in 90% of both AN and IN. Overall, 2/7/3 patients died due to both SCN. Sixteen of whom one occurred of haiploidentical HSCT related complication after of done for loss of response to G-CSF and the other of sepsis after the parents decided to stop G-CSF. From birth to the last follow up, a total of 108 infections occurred in 51/75 subjects (42%): 69 episodes in 12/12 patients with SCN, 25 infections were concentrated in 12/28 patients with AN and 14 episodes in 7/23 IN. Skin/subcutaneous infections (49%) and pneumonia (18%) were the most frequent localizations. Othamstisoiditis, tonsillar phlegmons, osteomyelitis (10%) and deep abdominals (5%) occurred almost exclusively in SCN. Microbiological characterization was possible in 28/108 episodes and showed a slight prevalence of Gram-negatives (13/26) over Gram-positives (9/26) without differences according to the type of neutropenia. Fungal infections was diagnosed in two different SCN subjects. G-CSF was used in all SCN patients, in 26% of AN and 4% of IN. In the whole population and in each group, the IR in the time-span from birth to the emergence of neutropenia was significantly higher vs IR calculated from the emergence of neutropenia to the last follow up (p<0.01). Summary/Conclusion. Among neutropenic children the highest infectious burden was observed in SCN, but was not negligible in AN and IN. Skin was the main involved site. Gram-negatives were the slightly prevalent microorganisms while fungi only occurred in SCN. The significant decrease of IR after the emergence of neutropenia vs previous period can probably be attributed to the increased awareness of the patients/family, to early antibiotic intervention and to the G-CSF therapy.
**SEVERE NEONATAL ALLOIMMUNE NEUTROPENIA IN A NEWBORN DELIVERED BY A CD16 DEFICIENT GYPSY WOMAN**

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**Background.** Neonatal Alloimmune Neutropenia (NAN) is an uncommon form of neutropenia of the newborn due to maternal IgG alloantibodies against fetal human neutrophil antigens (HNA) or leukocyte antigens (HLA). Inherited from the father but absent in the pregnant mother. The involved antibodies can readily cross the placental barrier, causing neutropenia in the newborn that can range from mild (1000-1500/µl) to severe (<500/µl). The incidence of NAN has been estimated to 1-1000/6000 live births, mainly due to HNA-1a,1b and HNA-2a antigens. Aim. We describe a severe case of NAN due to FcyRIIIb (CD16) alloantibodies occurred in a Roma (Gypsy) family.

**Patients and Methods.** A female child was born from second, uncomplicated pregnancy to a healthy 16-year mother, at the 40th week of gestation. Mother had a normal neutrophil count and the first child was healthy. Severe neutropenia (190/µl) and ocular infections, with otherwise normal laboratory findings, was detected on the first day of the newborn's life and persisted for 3 weeks. Direct (D) and indirect (I) granulocyte immunofluorescence tests (GIFT) were performed on newborn’s and mother’s blood samples. Flow cytometry cross-match of mother serum against father granulocytes was also performed. Family HNA-1, -3, -4 and -5 genotypes were evaluated using BAGene HNA-Type extra 3 kit (BAG Health Care). Anti-CD177 monoclonal antibody was used to determine HNA-2a phenotype. Results. A strong positive reaction was observed in the newborn’s D-GIFT. As reported in the table, family genotyping indicates that the HNA-1b antigen could be involved in this NAN case. Mother and newborn sera showed a strong reaction against father’s neutrophils and against 16 typed HNA blood donor neutrophils (including 6 HNA-1b subjects). Only one donor showing FcyRIIIb deficiency did not react with mother’s and newborn’s sera, indicating that the alloantibody specificity was not related to HNA-1b but to whole FyRIIIb. Interestingly, both mother and father resulted HNA-3a negative. Conclusions. Alloantibodies against FyRIIIb were the cause of this case of severe NAN developed in a CD16 null Gypsy mother. The described family had the HNA-3b phenotype that is quite rare in the Caucasian population. It would be interesting to determine the HNA frequencies in Gypsies.

**0820**

**HUMAN NEUTROPHIL ANTIGEN-3 (HNA-3) EXPRESSION IN BRAZILIANS**

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**Background.** Recently, it was characterized that the HNA-3 arises from a nucleotide polymorphism in the choline transporter-like protein 2 (SLC44A2), as a result of a single nucleotide polymorphism rs2288904. The HNA-3a and HNA-3b variants are encoded by a guanine and adenine at nucleotide position 461, respectively, resulting in either an arginine or a glutamine residue at amino acid position 154. The role of the HNA-3 is still unknown, but it is a target antigen in febrile transfusion reactions, immune neutropenias and transfusion-related acute lung injury (TRALI). Aims. To develop a technique of genotyping for HNA-3, and determine the prevalence of HNA-3a and HNA-3b in the Brazilian population. Methods. We obtained DNA of 500 healthy blood donors, 120 Amerindians, 59 Japanese individuals, and 124 patients with sickle cell anemia (SCA). All individuals were genotyped for rs2288904 by PCR-RFLP assay. The amplified product was digested with enzyme TaqI, specific to nucleotide guanine (HNA-3a). Genomic DNA of 9 blood donors (3 HNA-3a/a, 3 HNA-3a and 3 HNA-3 was sequenced to confirm the results of the PCR-RFLP. Results. The genotyping results showed that 66.2% of blood donors were homozygous for antigen HNA-3a, 30.2% were heterozygous (HNA-3a/b), while 3.6% were homozygous for antigen HNA-3b. All Amerindians typed homozygous for the antigen HNA-3a. Among Japanese subjects 45.6% were homozygous for HNA-3a, 43.6% were heterozygous, and 12.8% were homozygous for HNA-3b. Among African Brazilian individuals 72.6% were homozygous for HNA-3a, 25.8% heterozygous, while 1.6% were homozygous for HNA-3b. Overall, the frequency of the allele HNA-3a in the population of Brazilian blood donors was 0.80 and the allele HNA-3b was 0.20. The frequency of the HNA-3 was significantly different between blood donors and Japanese (HNA-3a - 66.2% vs 43.6%, p=0.0045; HNA-3b - 0.2% vs 77.4%, p=0.0005).
Aim. To evaluate the effect of the estrogen and progesterone on the oxidative burst of neutrophils, complement and hormonal regulation and these findings need to be investigated further in future.

Supported by EAPESP and CNPq.

Background. Neutropenia is defined as absolute neutrophil counts (ANC) <1,000/mm³ in infants and <1,500/mm³ in children. Neutropenia may be congenital or acquired with infection being the most common cause followed by autoimmune neutropenia (AIN) whether primary or secondary. Aim of this work was to study the prevalence, severity, and etiological causes of neutropenia in infants and children admitted to a University Children Hospital in Cairo, Egypt. Methods: 200 patients with neutropenia were recruited from the inpatients in The Children's Hospital Ain Shams University from April 1st 2010 to July 30th 2010, after having parental consent. Patients with a known hematological or immunological disease were excluded from the study. The patient's age ranged from 2 months-15 years (mean 24.4 ± 28 months), with male:female ratio of 1.1:1. Patients were classified according to ANC into mild, moderate and severe groups. Moderate and severe neutropenic children were followed for three months period. Follow up ANC was done till recovery or an underlying cause is uncovered. Viral serology was done for moderate/severe neutropenia patients including cytomegalovirus (CMV), Epstein-Barr virus (EBV), hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV. Anti-Neutrophil Cytoplasmic Antibody (ANCA) tested by enzyme immunoassay and bone marrow aspirate were done for prolonged moderate/severe neutropenia (persisting for 3 weeks or more). Results: Out of 200 patients, neutropenia was mild in 90 (45%) moderate in 56 (28%) and severe in 54 (27%). The clinical diagnoses among patients was bacterial pneumonia (38%), P. Jirovecii (17%), bronchiolitis (13%), urinary tract infection (9%), acute gastroenteritis (8%), hepatitis (6.5%), sepsis (5%), others (3.5%). All patients with mild neutropenia recovered within one week of follow up. Among the 110 patients with moderate/severe neutropenia, 36 (33%) recovered in <3 weeks, while 74 patients (27%) had prolonged neutropenia. Predictors of prolonged neutropenia were patient's age <18 months (P<0.01), ANC <500/mm³ (P<0.05), hemoglobin <10 g/dl (P<0.05) and positive CMV serology (P<0.05). Sex was equivocal. None of the patients with moderate/severe neutropenia had serological evidence of HCV, HBV, or HIV, while CMV serology was positive in 35 patients (43.5%) and EBV serology was positive in 8 patients (7.5%), all had severe neutropenia. ANCA was positive in 9 patients (aged 5-15 months), representing 30% of patients with prolonged neutropenia and 42.8% of prolonged severe neutropenia patients, all had hypercellular bone marrow with normal sequence of maturation. Conclusion. Neutropenia is a frequent finding in Egyptian infants and children, usually mild and transient, and mainly associated with infection. Cytomegalovirus and Epstein-Barr virus are important infections associated with prolonged moderate/severe neutropenia. Immune neutropenia is a common cause of moderate/severe neutropenia in the first two years of life. Close clinical and laboratory observation and adequate supportive care of the neutropenic child is of crucial importance.
mune abnormalities and risk to development of systemic lupus erythematosus (SLE). FcgRIla and FcgRlIib display functionally relevant genetic polymorphisms (H/R-131 and HNA-1a/b), respectively, which allelic variants can influence the biological responses and the susceptibility to and course of infectious diseases. In particular, the presence of the FcgRIla-R151 allele results in lower affinity binding to IgG2, a subclass of IgG specific for encapsulated bacteria. In addition, the homozygosis for FcgRIla-R131 and FcgRIIib-HNA-1b combination has been associated with impaired phagocytosis and the FcgR polymorphisms have also been implied in the susceptibility to and prognosis of infectious and autoimmune diseases. Aim. To assess the frequencies of the alleles for the FcgRIla and FcgRIIib polymorphisms and their combinations in Brazilian SLE patients compared to healthy subjects. Methods. Committee approval was obtained for the taking of blood samples and all studied patients agreed to provide them. All patients were diagnosed at Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP and fulfilled the American College of Rheumatology classification criteria for SLE (n=47). Ninety one healthy volunteers were included in this study as the control group. Genomic DNA was isolated from EDTA-anticoagulated blood using the salting out method. Determination of FcgRIla and FcgRIIib genotypes were performed using polymerase chain reaction (PCR)-based allotyping methods with allele-specific primers and the PCR products were analyzed by electrophoresis (2% agarose gel with gel red dye). Results. With respect to FcgRIla, the heterozygosis FcgRIla-HR-131 was the genotype most frequent in both SLE (53.2%) and healthy (45%) groups, being higher in SLE group. However, the frequency of the homozygosis H-131 was reduced in SLE (12.8%) compared to healthy groups. In SLE the homozygosis for HNA-1a was more frequent than that observed in controls, 26.7% and 16.5%, respectively; while the homozygosis for HNA-1b was more frequent in healthy (38.5%) group compared to SLE patients (28.9%). It was found the following frequencies for the alleles studied in SLE and healthy groups, respectively: H-131, 0.39 and 0.45; R-131, 0.61 and 0.56; HNA-1a, 0.49 and 0.39 and HNA-1b, 0.51 and 0.61. The most frequent genotype in SLE (44.4%) and healthy (45%) groups with an equal frequency in both groups. In SLE the homozygosis for HNA-1a was more frequent than that observed in controls, 26.7% and 16.5%, respectively; while the homozygosis for HNA-1b was more frequent in healthy (38.5%) group compared to SLE patients (28.9%). It was found the following frequencies for the alleles studied in SLE and healthy groups, respectively: H-131, 0.39 and 0.45; R-131, 0.61 and 0.56; HNA-1a, 0.49 and 0.39 and HNA-1b, 0.51 and 0.61. The most frequent genotype combination observed in SLE patients was HR-131/HNA-1a, 1b (24.2%) and HR-131/HNA-1b and R-131/HNA-1b in healthy group (17.4%). Summary. This study has implications and can contribute for the understanding of neutrophil abnormalities in SLE and identifying genetic markers that would predict patients who are at high risk for infections. clenimar@usp.br Supported by FAPESP and CAPES.
and Methods. Four hundred and forty nine MDS pts have been analyzed for demographics, stage and evolution during follow-up. Characteristics are presented in table 1. Most pts (335/449; 74.6%) were lower-risk MDS (<10% BM blasts), being refractory anemia and refractory cytopenia with multilineage dysplasia the more frequent subtypes. Karyotype was available in 350 pts, mainly those diagnosed from 2005. Results. Incidence rate of MDS pts increased over time: 1990-95: 66 (14.7%), 1996-2000: 59 (13%), 2000-2005: 138 (30.7%), 2005 to present: 172 (38.3%). Overall, 346 pts are dead (77.1%). For lower-risk MDS, 24 and 48-month survival were 56.9% and 42.8%, compared to 22% and 10.5% for higher-risk MDS (p<0.001). Variables associated with overall survival (OS) were (all p<0.001): percentage of BM blasts (<10% vs >10%), hemoglobin (>100 g/L vs <100 g/L), platelet (≥150x10^9/L, 50-150x10^9/L, <50x10^9/L) and transfusion dependency. Transfusion-dependent anemia was documented in 63.5% of pts at diagnosis. Supportive care including blood transfusion was the most common approach followed by observation until progression. Evolution to AML could be evaluated in 254 pts. 96/254 (37.7%) progressed to AML. Age at diagnosis (>75y) and BM blasts (>10%) were variables related to AML evolution. Causes of death were identified in 219 pts from medical charts and electronic records. Comorbidity was the main cause of death. Progression to AML (20.9%) and second neoplasia (9%) were identified as other major causes of death. Conclusions. Epidemiology of MDS remains unknown as many pts are underdiagnosed as observed by year of diagnosis. This analysis reflects preliminary approach to a large cohort of MDS pts from a tertiary center, with similar results to other registries. Depth of cytopenias seems to affect outcome together with classic prognostic factors. Detailed study for subgroups of pts and other registries. Depth of cytopenias seems to affect outcome together with classic prognostic factors. Detailed study for subgroups of pts and other registries.

### Table 1

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Survival at 12 Months</th>
<th>Survival at 24 Months</th>
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<tr>
<td>1990-95</td>
<td>66</td>
<td>56.9%</td>
<td>42.8%</td>
</tr>
<tr>
<td>1996-2000</td>
<td>59</td>
<td>22%</td>
<td>10.5%</td>
</tr>
<tr>
<td>2000-2005</td>
<td>138</td>
<td>30.7%</td>
<td></td>
</tr>
<tr>
<td>2005 to present</td>
<td>172</td>
<td>38.3%</td>
<td></td>
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</table>

### Table 2

<table>
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<tr>
<th>Variable</th>
<th>Lower Risk</th>
<th>Higher Risk</th>
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</thead>
<tbody>
<tr>
<td>Percentage of BM blasts</td>
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<td>&gt;10%</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>&gt;100 g/L</td>
<td>&lt;100 g/L</td>
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<tr>
<td>Platelet</td>
<td>≥150x10^9/L</td>
<td>50-150x10^9/L</td>
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### 0828

**LONG-TERM OUTCOME OF ISOLATED THROMBOCYTOPENIA ACCOMPANIED BY HYPOCHROMIC MORROW**

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**Background.** Most of the patients with isolated thrombocytopenia do not undergo bone marrow (BM) examination and a presumptive diagnosis of immune thrombocytopenia is made when the history, physical examination, complete blood counts, and examination of the peripheral blood smear do not suggest other etiologies. Hypochromic morrow without apparent etiologies in adults. Methods. We prospectively evaluated adult patients with isolated thrombocytopenia accompanied by hypochromic morrow without apparent etiologies in adults. Results. Between January of 2002 and December of 2006, a total of 224 patients with isolated thrombocytopenia were studied, including by BM examination. Among them, 20 patients (17 males and three females) were consistent with isolated thrombocytopenia accompanied by hypochromic morrow. The median age of the patients was 33 years (range: 18-79). At the initial presentation, the platelet counts range from 12,000 to 99,000/µL (median 65,000/µL) and were >50,000/µL in 16 patients (80%). BM cellularity ranged from 5 to 25% (median 15%) and was ≤10% in six patients (30%), 11-20% in 13 patients (65%), and 21-25% in 1 patient (5%). A weak positive correlation was present between the BM cellularity and platelet counts (R = 0.2044, P = 0.045). During the median 48-month follow-up range (range: 12-90 months), three of the 20 patients recovered platelet counts to normal levels (>150,000/µL) after 12, 56, and 66 months, respectively. Three patients developed pancytopenia after 11, 70, and 90 months, re-
spectively. Two patients were consistent with moderate aplastic anemia, and one was confirmed as refractory cytopenia with multilineage dysplasia. In the remainder of the patients, the platelet counts remained unchanged. Conclusions. Isolated thrombocytopenia accompanied by hypocellular marrow encompasses a group of heterogeneous conditions, and its subgroups represent the early manifestations of aplastic anemia/dysplastic syndrome or temporary BM depression of unknown etiology. Regular follow-up is therefore mandatory in patients with this condition and a large-scale observational study is warranted.

**0829**

**CYTOGENETIC FEATURES AND PROGNOSIS IN 496 ARGENTINEAN PATIENTS WITH PRIMARY MYELODYSPLASTIC SYNDROMES**

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**Background.** Myelodysplastic Syndromes (MDS) include a group of heterogeneous hematological disorders with variable risk of evolution to Acute Myeloid Leukemia (AML) and short survival. Around 40-50% of patients show abnormal karyotype at diagnosis. Recently, some isolated deletions have been related with a favorable outcome in MDS (i.e. 9q-, 11q-, 12p-) and Breems et al., 2008, proposed that monosomal karyotypes (MK) are an indicator of poor prognosis in AML. Aim. To characterize the cytogenetic profile, to test its prognostic value and to evaluate cytogenetic groups of risk in the Argentinean MDS population. Methods. This is a multicenter retrospective study of 496 primary Argentinean patients with primary MDS evaluated from 1984 to 2010 (including 259 patients from the Pilot Study and from the MDS Registry sponsored by the Argentinean Society of Hematology). Refractory cytopenia was defined according to Vallet et al., 2007 and patients were classified following FAB and WHO criteria. The median age was 69 (17-93) years with a male/female range of 1.3. The median survival was 50 months (2 months-50 years) with a male/female ratio of 1.5. During the follow-up (mean: 26 months), 111 (22.4%) evolved to AML and 225 (44.7%) died. Results. Gender, percentage of bone marrow blast, hemoglobin level, platelets count, number of cytopenias, LDH level and red blood cell transfusion requirements, FAB and WHO classification, IPSS and WPSS prognostic systems were significant predictive variables for prognosis (Kaplan-Meier and Long-Rank test, p<.001). Patients with normal karyotype (n=269, 55%, median survival: 50 months) had better outcome than those with cytogenetic abnormalities (n=207, 42%, 26 months, p=.001). Among abnormal karyotypes, 137 (66%) showed deletions and/or monosomies, 124 (59%) alterations (n=207, 42%, 26 months, p<.001). Among abnormal karyotypes, 137 (66%) showed deletions and/or monosomies, 124 (59%) involved, at least, one chromosome #5, #7, #8 and/or #20. The most common cytogenetic alterations were: -5/5q- (22% among cases with abnormal karyotype), -7/7q- (15%), -8 (21%), 20q- (9%) and -Y (8%). Karyotyopes were divided according to IPSS (p<.001) into Good (n=334, 67%, median survival: 48 months), Intermediate (n=102, 21%, 18 months). No significant differences were observed (n=54, 11%, 28 months), chromosome 7 alterations (n=19, 4%, 15 months), MK (n=30, 6%, 16 months) and other complex karyotypes (n=15, 3%, 18 months). No significant differences were observed among MK and other Poor cytogenetic findings (median survival: 15 months, p=.592). Patients with isolated deletions showed a similar behavior than Good cytogenetic findings (p=.487) and a borderline better outcome than the rest of the Intermediate ones (p=.07). An intermediate prognosis was observed when karyotypes included a deletion + other alteration (n=15, 3%, 31 months, p=.525). Conclusions. Cytogenetic findings had a clear impact in our population. Results in the present series, the largest in Latin America, suggest that MK are indicators of poor prognosis whilst the presence of an isolated deletion (not including 7q-) would be a good cytogenetic finding. However, the wide spectrum of low frequency aberrations stresses the importance of large study groups.

**0830**

**MOLECULAR ANALYSIS OF RPS19 AND RPL5 GENES IN GREEK PATIENTS WITH DIAMOND BLACKFAN ANEMIA**

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**Background.** Diamond Blackfan Anemia (DBA) is a rare inherited disease characterized by congenital defective erythropoiesis. Patients present early in life with severe normochromic, macrocytic anemia, while about 50% of the patients have also congenital anomalies. Heterozygous mutations in 9 ribosomal proteins genes have been identified in ~50% of patients. More than 30% of these mutations are located in the RPS19 and RPL5 genes. Aim. To evaluate the clinical and hematologic phenotype, as well as the incidence of mutations in the RPS19 and RPL5 genes in Greek patients with DBA. Methods. A questionnaire for patient with DBA, requesting data on clinical and hematological phenotype was sent to all the pediatric hematologists, hematology and transfusion units in Greece. Informed consent was obtained. Genomic DNA was extracted from peripheral blood lymphocytes from the identified patients with DBA. Molecular analysis with PCR, ECMA (Enzymatic Cleavage Mismatch Analysis) and direct sequencing was performed allowing detection and characterization of disease-causing mutations. PCR primers were specifically designed to amplify the whole coding region and the flanking intron/exon junctions of RPS19 and RPL5 genes. Results. 17 Greek patients (7 females and 10 males, mean age: 11.4±11 years) with DBA phenotype were included in this study. Congenital anomalies in different organs, including craniofacial, upper extremities, hands, eyes, heart and kidneys were present in 71.4% of the patients. Regarding the clinical course of the patients, 4 are treated with steroids and 8 are on regular transfusions. One patient, who had been on regular transfusions, was transplanted at the age of 8 years from an HLA-matched unrelated donor. Four patients are lost to follow up. One patient developed thyroid cancer at the age of 46 years. 6 patients (35.2%) were identified to carry mutations to either the RPS19 gene (5 patients, or the RPL5 gene (3 patients). 2 of the 3 mutations detected in the RPL5 gene, c.C909G (p.Y130x) and c.197_198insA (p.Y66x) are novel. Conclusions. This is the first report on the clinical and genetic evaluation of DBA patients in Greece. We have observed similar frequencies of mutations in the RPS19 and the RPL5 genes but with higher frequency of physical malformations to the ones reported from other countries. Higher rates of transfusion dependency may be related to referral bias. The occurrence of thyroid carcinoma in an adult patient with DBA is worth-noticing.

**0831**

**MOLECULAR ANALYSIS OF SBDS GENE IN GREEK PATIENTS EVALUATED FOR SHWACHMAN-DIAMOND SYNDROME**

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**Background.** Shwachman-Diamond Syndrome (SDBS) is a rare autosomal recessive disorder resulting from loss-of-function mutations in the highly conserved Shwachman-Bodian Diamond Syndrome (SDBS) gene. As a multisystem disease SDBS is characterized by bone marrow dysfunction, exocrine pancreatic insufficiency, metaphyseal chon-
dysplasia, short stature and increased risk of neoplasms. SBDS gene, located in chromosome 7q11, is the only gene presently known to be associated with SDS. Roughly 75% of patients with SDS carry mutations resulting from a gene conversion event with the adjacent pseudogene, SBDSP. In a limited number (<10%) of patients who present with clinical indications of SDS no mutations in SBDS are detected. Aim: To evaluate the clinical and hematologic phenotype, as well as the identification of mutations in the SBDS gene in Greek patients with SDS.

Methods. 7 patients, including 2 sisters, (5 females and 2 males, mean age: 12.4±5.9 years), meeting the diagnostic criteria for SDS were screened for SBDS mutations. Genomic DNA was extracted from peripheral blood lymphocytes and molecular analysis was performed with PCR, ECMA (Enzymatic Cleavage Mismatch Analysis), RFLPs and direct sequencing was performed allowing detection and characterization of disease causing mutations. PCR primers were specifically designed to amplify the whole coding region (five exons) and the flanking intron/exon junctions of SBDS gene but not the SBDSP pseudogene. RFLPs used the Bsu36I and Accl enzymes for the detection of the two most common c.183-184 T>A and c.258+2T>C mutations respectively. Data on clinical and hematological phenotype were collected for each patient after informed consent was obtained.

Results. Evaluated patients had a significant heterogeneous clinical presentation. 4 patients presented with neutropenia, and 2 with thrombocytopenia. 1 patient had trilineal cytopenia, evident at birth, with 32% of her marrow progenitors carrying an iso (7q) chromosomal abnormality. This patient was successfully transplanted from a matched sibling donor. One patient had a stable clonal abnormality (46,X,del(8)(q24) (c24 ARROW RIGHT) qter) in around 35% of her myeloid progenitors. 80% of patients showed congenital abnormalities on X-rays affecting the bones and heart. Only one patient was receiving therapy with granulocyte colony-stimulating factor, due to chronic neutropenia. Pancreatic insufficiency was mild to severe, with only 2 patients requiring pancreatic enzymes replacement. Growth hormone was used in 2 patients. 4 patients were compound heterozygotes for the common 183-184 T>A>C and 258+2T>C mutations. One of those was a mosaic which explained her very mild phenotype, while another was heterozygous for the 183-184 T>A>C and homozygous for the 258+2T>C mutation. One patient was compound heterozygote for 258+2T>C and c.460-1G>A novel mutation. Finally, one patient was compound heterozygous for both the common and the rare mutation. 4 patients presented with chronic transfusion therapy, to assess the extent of iron overload and to gain insight into patterns of care surrounding the use of iron chelation therapy in different geographical regions. Methods. TORS is a prospective, multinational, non-interventional study to collect information from patients >2 years of age requiring chronic transfusion therapy with newly diagnosed anemias (<12 months from diagnosis), including Low and Intermediate-1 megaloblastic syndromes (MDS), aplastic anemia (AA), Diamond-Blackfan anemia (DBA) and other transfusion-dependent anemias. Patients were recruited from Turkey, Russia and South Africa as well as from countries within the Asia-Pacific and Middle East regions. Patients with secondary or therapy-related MDS; Intermediate-2 or High-risk MDS; or acute leukemia were excluded. At the time of patient inclusion (baseline), patient demographics and details of iron overload and iron chelation therapy use were assessed.

Results. 559 patients recruited into the registry, the majority of patients had a diagnosis of MDS (58.5%, n=317), followed by AA (31.9%, n=172), DBA (1.1%, n=6) and other anemias (5.9%, n=32). The highest numbers of patients were recruited from Turkey (23.7%, n=128), China (18.0%, n=97) and Thailand (15.4%, n=83; Figure 1). The mean age (+SD) of the patients was 51.2 ± 25.7 years (range 2-92); 49.0% (n=264) were male and 50.3% (n=271) were of Asian origin. At study entry, the percentage of patients receiving transfusions ranged from 47.0-100.0%. The numbers of patients assessed with iron overload ranged from 4.1% (4/97) in China to 38.5% (5/13) in South Africa. The numbers of patients receiving iron chelation therapy ranged from 2.4% (2/83) in Thailand to 26.6% (34/128) in Turkey (recordings of iron overload and iron chelation therapy do not have to apply to the same patients).

Fig 1.

Figure 1.

Summary/Conclusions. The data suggest that iron overload and iron chelation practices vary between countries in these newly diagnosed patients with transfusion-dependent anemias. These analyses are limited by the small numbers of patients in some countries and by a potential bias towards recruitment of patients with specific anemias. These preliminary data nonetheless provide a better understanding of disease-management practices of newly diagnosed patients with anemia across different geographical regions.
0833

PEdiatric diagnosis of PaRoxYSmaL noCTurnal hemoglobinuria in the International PNH Registery

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*Cleveland Clinic Foundation, Cleveland, United States of America
*Hospital Saint-Louis, Paris, France
*Royal Melbourne Hospital, Parkville, Australia
Johns Hopkins University Medical Center, Baltimore, United States of America
Children’s Hospital of Philadelphia & Univ of Pennsylvania School of Medicine, Philadelphia, United States of America
*Duke University Medical Center, Durham, United States of America
*Osaka University Graduate School of Medicine, Osaka, Japan
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Background. Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematopoietic stem cell disease which can lead to life-threatening complications including intravascular hemolysis, thrombotic events (TE), and kidney disease. Improvements in the understanding and utilization of high sensitivity diagnostic tests and availability of targeted terminal complement blockade treatment have led to improved awareness and prognosis of PNH. There has been little systematic research focused on pediatric patients diagnosed with PNH due to small patient numbers. Aims. To describe the clinical characteristics of patients diagnosed with PNH as children and to compare these characteristics to patients diagnosed as adults in a large, international, observational disease registry. Methods. Data from 153 clinical sites in 19 countries on 5 continents (as of 31 Jan/2011) were analyzed to evaluate clinical presentation of PNH in patients first diagnosed at age ≤18 years (pediatric patients N=79) and at age >18 years (adult patients N=666). Baseline demographics, laboratory values (including flow cytometry), PNH-related medical history (including TE and kidney disease), and physician-reported PNH symptoms were compared for pediatric and adult patients. Patients (or guardians) gave informed consent prior to enrollment. Analyses were also stratified by percent GPI-deficient granulocytes (clone size) at registry enrollment (<50%, >50%). Results. Of the 79 patients diagnosed as children, 25 (32%) were still <18 years old at study enrollment. Approximately half of pediatric and adult patients were female. Years from disease start to enrollment was significantly higher for pediatric than adults (mean ± SD: 10.7 ± 11.6 vs. 7.8 ± 8.4, respectively, p=0.006). Overall, patients diagnosed with PNH as children were similar to those diagnosed as adults. There was no difference between pediatric and adult patients for underlying bone marrow disorders (59% vs. 43%), clone size (mean 63% vs. 64%), LDH fold above normal upper limit (mean 5.4 vs. 3.0), history of TE (10% vs. 12.2%, p=0.07), history of renal impairment (10% vs. 13%) or use of transfusions prior to registry enrollment (33% vs. 41%). Physician-reported hemoglobinuria and abdominal pain were comparable between pediatric and adult patients (54.5% vs. 56.8% and 44.3% vs. 36.9%, respectively). Pediatric patients had higher total red blood cell counts (mean 3.5 vs. 3.1±0.12/L, p<0.001) and less fatigue (52% vs. 72% p<0.001) than adults. Among patients with clone size ≥50%, pediatric patients were less likely to have a history of TE (7% vs. 22%, p=0.02) and less likely to have a history of fatigue (61% vs. 78%, p<0.01) but more likely to have headaches (54% vs. 36%, p=0.02) than adults. Pediatric patients were more likely than adults to receive a bone marrow transplant during registry follow-up (5.1% vs. 1.5%, p=0.03). Summary/Conclusions. PNH patients diagnosed in a pediatric setting present with similar clinical characteristics to adults such as LDH levels, hemoglobinuria, abdominal pain, underlying bone marrow disorders, TE and history of renal impairment. Among patients with clone size ≥50%, pediatric patients had less history of TE than adults, although TE is still an issue for pediatric patients. New clinical sites and geographic regions are encouraged to participate in the Registry (pnhregistry@iconpl.com).

0834

Deferredoxirx represenTs an eFfective oral iron chelator in low or intermediate-1 risk patients with MDS - a comparatIve study to the treatmenT with deferiprone

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Aims. An efficiency of different oral iron chelators was studied in a group of 113 MDS patients with <10% of bone marrow blasts. Patients and Methods. Sixty-five patients were treated with oral iron chelator deferiprox® (Exjade®). Median initial serum ferritin level was 2677.5 µg/l (range 780-9923 µg/l), the daily dose of the drug ranged from 10 to 40 mg/kg. Median duration of chelation treatment was 15.7 months (range 4-56 months). The results were compared to previously studied efficiency of deferiprone (Ferrprox®) in 48 patients with the same subgroup of MDS. Results. Chelation was effective (maintained or decreased iron stores) in 85% of patients (in 85% of 25 patients with serum ferritin ≤ 2000 µg/l and in 80% of 40 patients with serum ferritin 2001-5000 µg/l). Incidence of adverse effects was 56.9% and led to discontinuation of deferiprox treatment in 6% of patients. GIT symptoms represented the most frequent adverse effect (53.8% patients) that limited an effective escalation of the daily dose of the drug. An increase in serum creatinine level was observed in 20% of patients, after decrease in daily dose or transient discontinuation of the drug serum creatinine level stabilized in all patients. Skin rash was present in 4.6% as well as oedema and weight increase. When compared to our previous study with deferiprone, deferiprox effectivity was similar to that of deferiprone in patients with serum ferritin ≤ 2000 µg/l (85% vs. 76% responding patients with deferiprone) but significantly higher in those with serum ferritin > 2000 µg/l (80% responders v.s. only 46% treated with deferiprox). Incidence of adverse effects was similar (56.9% v.s. 62.5% after deferiprox), and GIT symptoms were the most frequent events after treatment with both the drugs. However, symptoms of deferiprox toxicity were mild and mostly transient and no myelosuppressive effect related to the drug administration was observed in contrast to deferiprone where drug related granulocytopenia occurred in 17% of patients and the treatment had to be discontinued in 29% of patients. Conclusions. Deferiprox administered in a daily dose of 15-40 mg (depending on initial serum ferritin level) represents an effective and safe treatment for iron overloaded patients with early phase of MDS without excess of blasts.

0835

myelodysplastic syndromes (MDS): does the hepatitis C virus (HCV) infection play a role?

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HCV infection plays a well-documented role in the aetiology of malignant lymphomas. HCV-infected persons are more likely to have low neutrophil and platelet count; some researchers support the hypothesis that HCV infection can itself cause cytopenias, but the mechanism is still unclear. Only a few studies have investigated the relationship between HCV and MDS, and the reports are contradictory. This research is aimed at evaluating the prevalence and clinical features of HCV infection in a population of MDS patients with a monocentric, retrospective analysis. One hundred twenty four patients, 84 males and 40 females, have been studied, mean age 76 years old (range 54-93), mean follow up 51 months (range 3-158); all underwent a complete blood cell count, bone marrow biopsy, peripheral blood smear examination, and were tested for HCV-antibodies, HIV-antibodies and serum hepatitis B virus (HBV) HbsAg antigen. Cytogenetic analysis has been performed in 98 patients. Clinical outcomes were evaluated using Kaplan-Meier analyses. According to the WHO classification, 31 cases were classified as refractory anemia (RA), 26 as refractory cytopenia with multilineage dysplasia (RCMD), 11 as refractory anemia with ringed sideroblasts (RARS), 16 as refractory anemia with excess blasts (RAEB-1), 16 as RAEB-2, 3 as MDS unclassified (MDS-U), and 19 as chronic myelomonocytic leukaemia (CML). All patients were nega-
tive for HIV infection; only 2 patients were HbsAg positive (1 in the AR group and 1 in the RAEB-2 group, respectively). Nineteen patients (15%) out of 124 were HCV positive; 12 out of 19 HCV positive patients were diagnosed with chronic hepatitis and 5 with mixed cryoglobulinemia. None of them was on antiviral treatment. Noteworthy, the HCV prevalence was significantly different among the WHO subtypes, as follows: RA=3.3% (1 out of 31), RARS=0% (0 out of 11), RCMD=14.3% (4 out of 28), RAEB-1=0% (0 out of 16), RAEB-2=20% (0 out of 16), CMMML=26% (5 out of 19), MDS U=0% (0 out of 5). The median overall survival (OS) was 49 months (mo) for RA patients, 71 mo for RARS, 25 mo for RCMD, 21 mo for RAEB-1, 9 mo for RAEB-2, 36 mo for CMMML patients, respectively. In the WHO subgroups with the highest HCV prevalence, no significant difference between HCV positive and HCV negative patients has been found either for OS (RCMD: p=0.82; RAEB-1: p=0.41; CMMML: p=0.36) or leukemia free survival (LFS) (RCMD: p=0.93; RAEB-1: p=0.19; CMMML: p=0.76). Conclusions. 1) In this experience the HCV prevalence rate in MDS patients (15%) was slightly higher than the values reported in the general population of comparable age in this geographic area (7-9%); 2) the HCV positive MDS patients are clustered in the WHO subgroups RAEB-1, CMMML, and RCMD; 3) in those WHO subgroups the HCV infection has no prognostic significance; 4) the HCV positive cytopenic patients require a careful evaluation for possible MDS.

**0836 PROLONGED HEMATOLOGIC AND MOLECULAR RESPONSE AFTER A LIMITED NUMBER OF AZACITIDINE CYCLES IN LOW-RISK MYELODYSPLASTIC SYNDROMES**

C Finelli,1 C Clissa,1 M Follo,2 D Russo,3 C Filì,3 A Curti,1 S Paolini,1

Prolonged hematologic and molecular response (RCMD: p=0.82; RAEB-1: p=0.41; CMML: p=0.36) or leukemia free positive and HCV negative patients has been found either for OS (mo for RARS, 25 mo for RCMD, 21 mo for RAEB-1, 9 mo for RAEB-2, 36 mo for CMMML patients, respectively. In the WHO subgroups with the highest HCV prevalence, no significant difference between HCV positive and HCV negative patients has been found either for OS (RCMD: p=0.82; RAEB-1: p=0.41; CMMML: p=0.36) or leukemia free survival (LFS) (RCMD: p=0.93; RAEB-1: p=0.19; CMMML: p=0.76). Conclusions. 1) In this experience the HCV prevalence rate in MDS patients (15%) was slightly higher than the values reported in the general population of comparable age in this geographic area (7-9%); 2) the HCV positive MDS patients are clustered in the WHO subgroups RAEB-1, CMMML, and RCMD; 3) in those WHO subgroups the HCV infection has no prognostic significance; 4) the HCV positive cytopenic patients require a careful evaluation for possible MDS.

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most severely affected patients have neutrophil counts of less than 200,000 per micrometer, platelet count of less than 20,000 and reticulocyte counts of less than 60,000 per cubic micrometer along with marrow cellularity of less than 25%. Immunologic events that precede the destruction are less clear. Involvement of the lymphocytes of CD34 or helper cells has been inferred from the over expression of the class II histocompatibility antigens. HLA B27 is a strong association with HLA antigens. Aims and Methods. Total 315 patients with the confirmed diagnosis of Aplastic anemia were registered in our center from April 1996 till April 2006 and their detailed assement was performed. After the initial counseling of the illness and symptomatic treatment; these patients were offered the ultimate treatment considering Allogenic BMT as first line treatment option. HLA typing was performed of only those patients who were fulfilling the criteria of bone marrow transplantation. Later on results of HLA antigens were analyzed. Results. Total 318 patients were enrolled out of which 237 were males and 71 females. 131 patients belonged to pediatric age group (under 15) and 171 above that. The mean age of presentation was 20.79, while the median was 17.21% of the patients under went BMT, 22% patients were given cyclosporin and 5% received ATG with or without cyclosporin. In HLA typing results, there was strong association with HLA B5 (52). Conclusion. Aplastic anemia has about 3-4 fold higher incidence in Asian population. Although these results reflect the number of cases from only one tertiary care center, but still the magnitude of disease can be roughly estimated. Further studies are needed to establish other causal factors like occupational history and drugs history of last 6-8 months period of time, so that further details regarding this neglected illness could be explored.

0840 EFFICACY AND TOLERABILITY OF 5-DAY AZACYTIDINE DOSE INTENSIFIED REGIMEN IN HIGHER RISK MDS

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Background. Azacytidine is a hypomethylating agent indicated for treatment of higher risk Myelodysplastic Syndromes (MDS). A recently published phase III trial demonstrated improved overall survival (OS) of MDS patients treated with azacytidine compared to those receiving conventional care regimens, thus establishing this treatment option as first line therapy in those patients for whom bone marrow transplantation is not an option. Aims. Evaluate the efficacy of azacytidine regimen in terms of transfusion independence (TI), overall response (OR), time to AML transformation and tolerability in patients with higher risk MDS and AML with 20-80% blasts. Methods. Higher risk (International Prognostic Scoring System - IPSS INT-2 and high risk) MDS patients were treated with azacytidine. Patients eligible for treatment. OR, including complete response (CR) and partial response (PR) and TI, defined according to the 2000 International Working Group Criteria (IWG), were assessed by blood and bone marrow examination. Treatment cycles were repeated until toxicity or disease progression. Results. A total of 48 patients were treated with azacytidine between June 2006 and September 2010; 47 of these, only 26 were treated for at least 4 cycles. Mean age was 65 years old (range 85-36) and male sex was predominant (M:F of 1.3). Eleven patients had refractory anemia with excess blasts (RAEB), six had secondary AML, five had chronic myelomonocytic leukemia (CMMI), two had refractory cytopenias with ringed sideroblasts and two had acute erythroid leukemia. Most patients were high risk according to IPSS scoring (78%). Azacytidine was used as first line therapy in 35% and as second line in half. An average of 8 cycles (4-22) per patient were administered. The transfusion independence rate was of 50%, with average response duration of 6.5 months. Overall response rate was 51% (7 CR and 14 PR). During the follow-up period, fourteen patients died. Five patients showed disease progression to AML; four of them had never shown any response to azacytidine, while the other one had obtained transfusion independence. Overall survival (OS) from diagnosis was of 26 months, while OS from beginning of treatment was 22 months. There was limited toxicity, mainly grades I and II gastrointestinal and skin toxicity. Eleven patients (42%) had grade III hematological toxicity and four (15%) suffered Grade IV haematological toxicity. Conclusion. The efficacy of azacytidine in achieving TI and prolonging survival in MDS is well recognized. In this study, azacytidine improved quality of life and overall survival regardless of the quality of response. Treatment was well tolerated, with limited toxicity. Our results, though coming from a small group of patients, were comparable to those reported in the literature.

0841 CLINICAL IMPACT OF UNCONTROLLED COMPLEMENT ACTIVITY IN JAPANESE NON-TRANSFUSED PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Introduction and Aims. Paroxysmal nocturnal hemoglobinuria (PNH) is a debilitating and life-threatening hematopoietic stem cell disorder...
characterized by chronic uncontrolled complement activation. PNH evolves from the clonal expansion of hematopoietic stem cells with complete or marked loss of the terminal complement inhibitors CD55 and CD59, consequently rendering red blood cells susceptible to systemic lysis. This chronic hemolysis underlies the severe morbidities and mortality associated with the disease: life-threatening thromboembolism (TE), chronic kidney disease (CKD), pulmonary hypertension and organ damage, fatigue, abdominal pain, dysphagia and dyspnea. While many patients with PNH have anemia, low hemoglobin levels and transfusion requirements may not adequately reflect the burden of disease due to hemolysis since these parameters are influenced by other factors unrelated to hemolysis or complement activation including bone marrow dysfunction, patient specific factors and physician clinical assessment. Here, we report a paucity of reported data in Japanese PNH patients who are not transfused that sufficiently captures their burden of disease. Here, we report the efficacy response of two patients recently treated with eculizumab in the AEGIS study who received no blood transfusions in the 2 years prior to study participation. Methods. Eculizumab was administered in 29 Japanese PNH patients (14 men and 15 women; median patient age, 47 years; range 26-70 years) at 9 institutions in Japan for 12 weeks in the AEGIS study. This report summarizes the evaluation of two patients with history of no blood transfusions that enrolled and were treated in the AEGIS study. Results. At baseline, the two patients who had received no blood transfusions prior to study participation were hemolytic (LDH approximately 7 and 11 fold above normal), demonstrated significant organ damage with evidence of thrombosis (1 patient with DVT) and renal disease (CKD stage 2 and 1), and suffered due to declining quality of life as measured by FACT-fatigue and EORTC-QLQ C-30 scores. In both patients, eculizumab treatment resulted in substantial 78%-88% reductions in LDH, significant improvements in fatigue as measured by FACT-fatigue (changes of 5 and 33 points; >3-point improvement is clinically meaningful), improvement in dyspnea in one patient (change of 33 points from baseline; ≥10 point improvement is clinically meaningful), and elimination of CKD with no subsequent thrombotic events in both patients. The LDH reduction in these non-transfused patients compares favorably to the 87% reduction in LDH observed in the entire AEGIS patient population. Conclusion. These data demonstrate that despite a history of no transfusions, at baseline these non-transfused PNH patients had significant clinical evidence of disease burden including chronic hemolysis, history of thrombosis, renal disease, and impaired quality of life. These findings underscore the central role of uncontrolled complement activation and the resultant sequelae associated with hemolysis, rather than anemia or transfusion requirements, in guiding the treatment of patients with PNH. Further, inhibition of terminal complement activation with ecilizumab in patients with PNH improves hemolysis, fatigue, dyspnea and other significant morbidities of disease whether or not they had received transfusions prior to participation in the AEGIS trial.
QUALITY ASSESSMENT OF INTERNET MORPHOLOGY EDUCATION PROJECT BASED ON VIRTUAL MICROSCOPY

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Background. Availability of new technologies has expanded the possibilities for education of microscopic morphology on internet. Among others virtual microscopy has enabled evaluation of whole microscopic smears from peripheral blood or marrow aspirate up to a significant magnification. Aims. To assess the quality of virtual microscopy internet project used for pregraduate education of medical students. Access to the project is currently restricted only to students and members of project team at www.e-hematologie.cz. Methods. Seven experts from the Czech Republic, Finland, Germany and Italy have evaluated the pictures of single normal and pathologic cells and full scope scans of marrow or blood microscopic smears obtained by CellVision DM 96 (Sysmex) and dotSlide system (Olympus). Results. 94 categones of normal and pathologic cells were randomly tested by 3 to 5 experts on erythroblasts, promyelocytes, unmature eosinophiles and basophiles; by 4 experts on quality of special staining. Experts agreed on the need to exchange the provided picture or scan for a new one because of poor selection in these cases of: mastocytes (6 of 7 experts); prolymphocytes (4/7); monoblasts, promonocytes, megakaryoblasts, micromegakaryocytes (3/7). A better selection of scanned smears was suggested by 3 experts in cases of APL, AML with inv16, MDS and acute monoblastic leukemia. Scans more than one case of HCL and chronic prolymphocytic leukemia should be provided. Conclusions. A very good level of agreement between the authors of the project and external auditors was achieved. Modern tools enabling virtual microscopy have been confirmed as excellent or very good for education of morphology (agreement among all experts). Carefull selection of the smears and appropriate use of technologies may avoid scans and pictures of inadequate quality. Implementation of unified morphology classification and links to atlasional and European and American projects are mandatory to further improve the quality of the project. The use of these ICT tools is nowadays essential for a consensus morphological diagnosis, that is mandatory in those hematological malignacies where the identification and enumeration of abnormal cells still remain mandatory for the diagnosis.

LONG-TERM OUTCOMES AFTER IMMUNOSUPPRESSIVE TREATMENT FOR MODERATE APLASTIC ANEMIA

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Background. The clinical course and current management of acquired moderate aplastic anaemia (MAA) are variable and few data concerning long-term results of immunosuppressive therapy (IST) are available. Aims. To evaluate of IST efficacy and long-term outcomes in patients with MAA. Methods. We analyzed the long-term outcome of 59 patients with MAA (28 M and 31 F; age 6-65 years, median 25) treated with ATG and CsA (n=41), including repeated courses in 14 patients) or with CsA alone (n=18) in two centers between April 1994 and February 2011. MAA was defined as hypocellular bone marrow and at least bi-lineage cytopenia lasting more than 4 weeks without meeting the criteria for severe AA. Results. A total of 46 patients (78 %) responded to IST but only 10 patients (17 %) achieved complete response. Eleven patients (24 %) relapsed and 9 responded again after retreatment with ATG or/and CsA. Late events included MDS/AML (n=5), rectal cancer (n=1) and hemolytic PNH (n=2). There were 1 early and 5 late deaths. With a median follow-up of 38 months (range 1-236), the probability of 5-, 10- and 15-year overall survival were 85.8 ± 3.7 %, 79.7 ± 8.9 % and 66.2 ± 16.1 % respectively. Due to high incidence of relapse and late events the failure-free survival was much lower: 52.2 ± 8.2 % and 32.3 ± 8.6 % at 5 and 10 year respectively with no apparent plateau in the curve. Conclusions. These data demonstrate that despite encouraging short-term results the long-term prognosis of MAA after IST is rather unpredictable. Longer follow-up in a larger cohort and further studies into biologic heterogeneity are warranted to determine optimal treatment strategy in MAA. Careful monitoring of hematologic response would be required in order to clarify the role and timing of allogeneic bone marrow transplantation.
**Myeloma and other monoclonal gammapathies - Biology 2**

**0846 INSULIN GROWTH FACTOR BINDING PROTEIN-3 EXPRESSION AND ITS PROGNOSTIC SIGNIFICANCE IN MONOCYTONAL GAMMAPATHIES**

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**Background.** Multiple myeloma (MM) is a clonal malignancy of plasma cells characterized by several genetic and epigenetic aberrations. IGFBP-3 gene is a member of the insulin-like growth factor binding protein (IGFBP) family and it can regulate cell growth and death by its ability to bind insulin-like growth factors (IGFs) as well as its IGF-independent effects involving binding to other molecules. Its role in carcinogenesis in other tumors is still debated. Previous studies demonstrated that in patients with MM the levels of IGFBP-3 protein were decreased. We have recently shown that the gene expression of IGFBP3 in myeloma cell lines were decreased. **Aim.** We analyzed the gene expression of IGFBP3 samples from patients with monoclonal gammapathies at diagnosis and we evaluated the correlation between IGFBP3 gene expression levels and overall survival of patients in order to determine the clinical relevance of this gene. **Methods.** 128 samples of patients at the moment of diagnosis (14 MGUS and 114 with MM) were retrospectively evaluated. The diagnosis was based on standard criteria. IGFBP3 mRNA expression was measured in each samples by real-time PCR using TaqMan Gene Expression Assays and the 7900HT Real-Time PCR System (Applied Biosystems Foster City, CA). **Results.** 128 patients; median age 76; female 52; median age 68 years (range 40-89). 125 stage: stage I 41%, stage II 38%, stage III 26%. In 79/128 (57%) patients we found lower levels of IGFBP3 expression compared to the calibrator sample, the remaining 55/128 (43%) patients showed increased level of gene. We analyzed the correlation between overall survival and IGFBP3 levels and we surprisingly observed that patients with lower levels of the gene had a significantly better overall survival (p 0.0215). **Conclusion.** These results suggest IGFBP3 down-regulation as a good prognostic factor. Further analysis of correlation of IGFBP3 gene expression with clinical and biological characteristics in these MM patients is ongoing. More studies are needed to better understand the role of IGFBP3 in myeloma pathogenesis.

**0847 NEURAL STEM CELL MARKER NESTIN AS A POTENTIAL UNFAVORABLE FACTOR FOR MULTIPLE MYELOMA**

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**Background.** Neural stem cell marker nestin is considered to be a characteristic marker of multipotent proliferative precursors with primitive and undifferentiated phenotype found in some embryonic and fetal tissues. Nestin expression has been also detected in many solid tumors and is proposed to be a suitable diagnostic and prognostic indicator of malignancy and a putative marker of cancer stem cells in solid tumors. Unexpectedly, our previous results confirmed nestin levels in mature CD138+ plasma cells (PC) of multiple myeloma (MM) patients by flow cytometry; significant differences were found between nestin levels in MM and individuals without any hematological malignancy. One third of MM patients had more than 50% of nestin-positive PC. Nestin seems to be a specific marker only for CD138+PC. Expression of stem/progenitor cell marker nestin might be a novel prognostic factor for MM. **Aims.** The aim of this pilot study was to analyze nestin expression in CD138+PC of MM and evaluate relationship between nestin expression and cytogenetic aberrations in CD138+PC of MM. **Methods.** A total number of 22 MM patients (12M/10F; median age 58 years) were included in this study. Nestin expression was evaluated as 2ΔCt of nestin gene by quantitative real-time PCR in 22 MM patients. As a calibrator was used commercial total bone marrow RNA from healthy donors. CD138+PC of MM patients were analyzed for del(13q14), del(17p53), IgH rearrangement (IgH), Igq21 gain and hyperdiploidy/non-hyperdiploidy (HY/non-HY) by interphase FISH. HY was assessed as trisomy of chromosome 5, 9 and 15. Differences of nestin expression between cytogenetic-aberration positive and negative patients were analyzed by non-parametric Mann-Whitney U test. **Results.** The whole group of MM patients had the median 2ΔCt 2.62 (range: 0.49-39.41). Statistical significant differences were found between IgH-positive: median 2ΔCt 5.42 (range: 0.49-39.41) and IgH-negative patients: median 2ΔCt 0.72 (range: 0.46-6.62), p=0.025; HY-patients: median 2ΔCt 0.72 (range: 0.46-24.44), and non-HY-patients: median 2ΔCt 7.00 (range: 0.62-39.41), p=0.024; trisomy 15-positive: median 2ΔCt 0.59 (range: 0.46-4.24) and trisomy 15-negative patients: median 2ΔCt 8.43 (range: 0.99-39.41), p<0.001. **Summary.** Our results confirmed that nestin expression is associated with IgH rearrangement. Our study was limited to IgH rearrangement and to HY patients. We suppose that nestin might be associated with unfavorable prognosis, since IgH rearrangement and HY are unfavorable prognostic factors. However, further studies are required to assess nestin role in multiple myeloma pathogenesis. Supported with research program MSM of Czech republic Nr. LC06027, P304/10/1395 and MSM 0021622434.

**0848 INDUCTION BY LENALIDOMIDE AND DEXAMETHASONE COMBINATION INCREASES CD4 AND CD8 REGULATORY CELLS OF NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS**

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**Background.** Naturally occurring regulatory cells play an active role in maintaining immune system homeostasis. In various hematological malignancies and solid tumors it was proved that T regulatory cells (Tregs) were increased and functionally active in abrogating immune responses. Few studies documented the expansion of Tregs in the relapsed multiple myeloma (MM) patients after lenalidomide and dexamethasone treatment. **Aims.** To screen the frequency of regulatory (CD4 and CD8 Tregs) and suppressor (Ts) cells between pre- and post-induction cycle by lenalidomide plus dexamethasone combination in newly diagnosed MM patients. **Methods.** Thirteen newly diagnosed MM patients were analyzed for cell frequency and function by ELISPOT assay. The patients were treated with a combination of lenalidomide and dexamethasone (20 mg IV weekly for 21 days every 4 weeks). Results were compared with those obtained in three healthy donors. **Results.** Significant increase in the number of regulatory T cells was observed in both CD4 and CD8 Treg subsets of MM patients. **Conclusion.** Our study suggests that the combination of lenalidomide and dexamethasone may modulate the balance in the regulatory T cell population of newly diagnosed MM patients.

**Figure 1.**
MM patients were included in this study. Median age of the patient cohort was 60 years (range: 50-64). Based on international staging system (ISS) patients were represented as: ISS1-4/13 (31%), ISS2-7/13 (54%), and ISS3-2/13 (15%). All patients were induced by lenalidomide plus dexamethasone combination for 4 cycles at 28 days interval [lenalidomide (25mg/1-21 days) and dexamethasone (40 mg on days 1,8,15, 22)]. One million of erythrocytes lysed peripheral blood (PB) cells were labeled with following Fluorochrome combinations: FoxP3-PE/ CD28-PE/ CD4-PerCy5.5/ CD8-APC/ CD25-PE-Cy7 and analyzed by multiparameter flow cytometry. We compared the PB frequency of CD4 Tregs, CD8 Tregs and Ts cells between pre-induction and post-induction cycles in all 13 patients. Ten age-matched healthy volunteers (HVs) PB samples were also analyzed for comparison. Results. CD4 Tregs were strictly identified as CD4+CD25hi+FoxP3+ and CD8 Tregs were characterized as CD8+FoxP3+. Ts cells were identified by absence of co-stimulatory molecule (CD8+CD28-). We observed similar frequency of CD4 Tregs in between MM patients and HVs (median (range) = 2.9% (0.9%-3.42%) vs. 3.0% (1.9%-3.59%); P=0.78). Whereas, for CD8 Tregs MM patients had significantly elevated level compared to HVs [CD8 Tregs median (range)= 0.32% (0.01%-1.21%) vs. 0.15% (0.04%-0.22%); P= 0.009]. Similarly to CD8 Tregs, Ts cells were also prominently increased in MM patients compared to HVs [CD8 Tregs median (range)= 54.86% (19.68%-71.25%) vs. 50.36% (12.77%-60.97%); P=0.031]. Post-induction cycle observations showed significant increase in CD4 Tregs compared to pre-induction (Table 1). For CD8 Tregs and Ts cells an increasing trend was observed in post-induction cycles compared to pre-induction, but statistical significance was not observed in first post-induction cycle for CD8 Tregs (Table 1). Conclusions. In summary, regulatory cells were increased after induction with lenalidomide and dexamethasone combination in newly diagnosed MM patients. This observation should be taken into consideration to efficiently improve the immuno-stimulatory effects for MM patients.

Table 1.

| Comparison of Pre- and Post-induction by Lenalidomide (lenalidomide (25mg/1-21 days) and dexamethasone (40 mg on days 1,8,15, 22). One million of erythrocytes lysed peripheral blood (PB) cells were labeled with following Fluorochrome combinations: FoxP3-PE/ CD28-PE/ CD4-PerCy5.5/ CD8-APC/ CD25-PE-Cy7 and analyzed by multiparameter flow cytometry. We compared the PB frequency of CD4 Tregs, CD8 Tregs and Ts cells between pre-induction and post-induction cycles in all 13 patients. Ten age-matched healthy volunteers (HVs) PB samples were also analyzed for comparison. Results. CD4 Tregs were strictly identified as CD4+CD25hi+FoxP3+ and CD8 Tregs were characterized as CD8+FoxP3+. Ts cells were identified by absence of co-stimulatory molecule (CD8+CD28-). We observed similar frequency of CD4 Tregs in between MM patients and HVs (median (range) = 2.9% (0.9%-3.42%) vs. 3.0% (1.9%-3.59%); P=0.78). Whereas, for CD8 Tregs MM patients had significantly elevated level compared to HVs [CD8 Tregs median (range)= 0.32% (0.01%-1.21%) vs. 0.15% (0.04%-0.22%); P= 0.009]. Similarly to CD8 Tregs, Ts cells were also prominently increased in MM patients compared to HVs [CD8 Tregs median (range)= 54.86% (19.68%-71.25%) vs. 50.36% (12.77%-60.97%); P=0.031]. Post-induction cycle observations showed significant increase in CD4 Tregs compared to pre-induction (Table 1). For CD8 Tregs and Ts cells an increasing trend was observed in post-induction cycles compared to pre-induction, but statistical significance was not observed in first post-induction cycle for CD8 Tregs (Table 1). Conclusions. In summary, regulatory cells were increased after induction with lenalidomide and dexamethasone combination in newly diagnosed MM patients. This observation should be taken into consideration to efficiently improve the immuno-stimulatory effects for MM patients. 

This study was supported by the respective grants- MSM0021622434, LC06027, IGA NS10406, IGA NS10408, and GACR P304/10/1395.

0849

ROLE OF THE PLATELET DERIVED GROWTH FACTOR-AB IN TUMOR GROWTH AND ANGIogenesis IN RELATION WITH OTHER ANGIogenic CYTOKines IN MULTiple MYELOMA

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Background. Angiogenesis is a complex process indispensable to growth, invasion and metastasis in various malignant tumors, including multiple myeloma (MM). Various angiogenic cytokines have been implicated in the angiogenic process. Among them, platelet derived growth factor-AB (PDGF-AB), has been reported to be potent stimulator of angiogenesis not only in solid tumors but also in haematological malignancies, including MM. Aims. The aim of the study was to investigate the relationship between PDGF-AB, microvascular density (MVD) and various angiogenic cytokines, such as basic-fibroblast growth factor (bFGF), angiogenin (ANG) and interleukine-6 (IL-6), in patients with newly diagnosed MM. Methods. Serum concentrations of the above cytokines were determined in 47 MM patients before treatment, in 22 in plateau phase, as well as in 20 healthy individuals. Serum PDGF-AB, bFGF, ANG and IL-6 measurements were performed by ELISA with a commercially available kit. We also performed bone marrow biopsies prior and after treatment, in plateau phase. MVD was determined by staining bone marrow vessels with anti-CD31. Results. Mean serum values of PDGF-AB and b-FGF were significantly higher in the group of MM patients in comparison to control group (p<0.001 and p<0.001 respectively). The mean serum values ±SD values of PDGF-AB and b-FGF were significantly higher with increasing stage of MM disease (p<0.001 and p<0.001 respectively). Significant positive correlations were observed between serum PDGF-AB, ANG and IL-6 levels with MVD (r=0.361 p<0.01, r=0.0876 p<0.001 and 0.342 p<0.01 respectively). Furthermore we found significant positive correlations between PDGF-AB and b-FGF, IL-6, ANG and B2M (r=0.324 p<0.02, r=0.491 p<0.0001, r=0.457 p<0.002 and r=0.365 p<0.01 respectively). We also found that patients with high MVD had statistically significant higher levels of PDGF-AB (p<0.017), when as cutoff point of MVD was used the median value 7.7. Furthermore significant difference was found between serum levels of PDGF-AB in pre and post induction. Serum ANG concentrations in the entire group of MM patients ranged from 246.40-1615.4 pg/ml with a mean ± SD of 669.6±382.5 pg/ml. These values were significantly higher than those found in the control group (p<0.001), which ranged from 105.78-356.63 pg/ml. Mean values for ANG in the group of MM patients were significantly higher with increasing stage of disease (p<0.001). Mean values of MVD were significantly higher in MM patients than in controls (p<0.0001). Among the stages in the entire group of MM patients MVD was significantly higher only in stage III in comparison to stage I (p<0.01). There was a difference in survival times between patients with high vs. low PDGF-AB levels (51 vs. 66 months) and high vs. low ANG levels (57 vs. 67 months), but the difference could not reach statistical significance in either case. In contrast, only in MVD groups survival time was significantly higher in MVD median group (76 vs. 51 months, p<0.02). Conclusions. Our results showed that there is a strong positive correlation between PDGF-AB and some angiogenic cytokines as well as with MVD, which indicates a role of PDGF-AB in the complex network of cytokines in inducing bone marrow neo-vascularization in patients with MM.

0850

LENALIDOMIDE TREATMENT POST AUTOLOGOUS TRANSPLANTATION FOR MULTIPLE MYELOMA DECREASES TERMINALLY DIFFERENCIATED CD4 AND CD8 T CELLS AND INCREASES NUMBER OF TREG

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Background. Treatment with Lenalidomide, a structural analogue of Thalidomide, plus dexamethasone (Dex) increases time to progression in relapse or refractory multiple myeloma. However, due to its pleiotropic effect, it’s not known if its efficacy is due only to a direct tumor toxicity or benefit also of its immunomodulatory effects. Aims. We wanted to assess in vivo the changes induced by Lenalidomide treatment on T-cell reconstitution in a cohort of multiple myeloma patients. Patients. Thirty-four myeloma patients were treated with the induction combination bortezomib plus Dex, followed by high dose melphalan (140-200 mg/m2) and an autologous transplantation with peripheral blood stem cells. Between 3 to 6 months post autograft, patients were randomized in 2 groups: 12 received 25 mg/day of Lenalidomide for 2 months, 3 weeks per month plus 40 mg of Dex, once a week; then 10 mg/day of Lenalidomide only until relapse. 22, a placebo only. Methods. T lymphocyte subpopulation percentage and absolute counts were assessed by multicolor flow cytometry from diagnosis until 18 month after autograft. Results. After Lenalidomide plus Dex treatment, we observed a significant decrease in percentage and absolute counts of CD4+ or CD8+, CD45RA+CCR7- effector T cell subpopulations. Surprisingly, CD4+CD25high or CD4+CD25-low Treg increased significantly more in treated patients. No correlation was found with documented infections, relapse or survival. Conclusions. These data suggest that, in vivo, in Human, Lenalidomide plus Dex efficacy on Myeloma tumor is not T cell mediated but this treatment could have a
negative impact on T cell immunosurveillance. Additional studies are required to better assess the respective effects of Lenalidomide and Dex on immune function.

0851
METAPHASE INDUCTION IN MULTIPLE MYELOMA: A NEW CYTOGENETIC APPROACH FOR G-BANDING ANALYSIS
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Background. The usefulness of conventional cyto genetic analysis for the classification and prognostication in acute and chronic leukemia has been demonstrated. In multiple myeloma cytogenetic abnormalities also play an important role as prognostic factor. However, classical cyto genetic fail to detect these abnormalities because the low mitotic index of plasma cells in vivo. The analysis of a set of subjects for the most commonly known aberrations is usually done by FISH on interphase cell. Aims. The goals of this investigation were the use of the oligonucleotide DSP30 in combination with IL-2, IL-6 and IL-10, as a B cell mitogen for cytogenetic investigation in MM and the comparison between the karyotype analyses obtained (G-banding) with FISH profile of cells. Methods. For metaphase induction, bone marrow mononuclear cells of 55 patients with MM were cultured in RPMI 1640 medium with 20% fetal calf serum in the presence of the immunostimulatory CpG-oligonucleotide DSP30 and IL-2/IL-6/IL-10 for 72h. Additionally, two extra set of cell culture was performed for each patient with the same stimulant agent (G-banding analysis, when possible) and FISH. The FISH panel included probes for the detection of translocations involving IGH gene (14q32), gains associated to 11q23-25 and 1q21 and deletion of 13q14. The cut off levels for IGH translocations was (>3.2%), gains of 11q23-25 and 1q21 (2.4% and 2.7%, respectively) and deletion 13q14 (2.5%). All cut off levels were established based on the karyotype patterns observed in a group of 12 age and sex-matched normal control peripheral blood samples studied with the same probes. Results. In the cells treated with stimulus, the cytogenetic analysis was possible in 42 patients (76.5%). On the other hand, in the group without any stimulus, the cytogenetic profile was successful in 20 patients (36.4%), being 8 (40%) with normal karyotype and 12 with chromosomal abnormalities (60%). The group that received stimulus, normal karyotype was found in 16 patients (38%) and metaphases with abnormal karyotype were seen in 26 subjects (62%). Among the cytogenetic profile obtained in both groups were observed aneuploidies involving the chromosomes +3, +5, +9, -13, -14, +15, +16, -18, +19, +20, -22 and structural abnormalities such as add(1)(p12), inv(3)(q21q26), del(13)(q14), add(14)(q32) and t(14;16)(q32;q23). FISH analysis performed in those patients whose bone marrow cells were not stimulated displayed the same chromosomal abnormalities as identified in the group with stimulus. Conclusion. These results indicate that the addition of the immunostimulatory oligonucleotide DSP30 in combination with IL-2, IL-6 and IL-10 showed to be effective to induce cell cycle progression of MM cells in vitro.

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THE ASSESSMENT OF PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) AND ITS RELATIONSHIP WITH PROINFLAMMATORY CYTOKINES AND PARAMETERS OF DISEASE ACTIVITY IN MULTIPLE MYELOMA PATIENTS
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Background. Multiple myeloma (MM) is a malignant disease of plasma cells that localize to the bone marrow (BM). Clonal plasma cells and BM stromal cells produce several proinflammatory cytokines that are involved in the pathogenesis of the disease. Aims. The aims of the study were to investigate circulating levels of proinflammatory cytokines such as Interleukine-1β (IL-1β), Interleukine-6 (IL-6), Interleukine-8 (IL-8), Macrophage Inflammatory Protein-1α (MIP-1α) as well as LDH, B2 microglobulin, in MM patients in treatment, and to discuss its significance in tumor progression. Additionally we analyzed the correlation of measured mediators with the proliferative activity assessed by proliferating cell nuclear antigen (PCNA) immunohistochemistry staining. Methods. 44 newly diagnosed MM patients and 20 healthy controls were studied. Patients were staged with Durie-Salmon system. Serum samples were collected at the diagnosis. We also performed BM biopsies prior to treatment. We determined serum levels of IL-6, IL-1β, IL-8 and MIP-1α using enzyme-linked immunosorbent assay (ELISA), as well as PCNA expression in the BM. Results. The mean concentrations of the measured cytokines IL-6, IL-1β, IL-8 and MIP-1α in the entire group of patients were 5.8±2.9 pg/ml, 2.8±1.3 pg/ml, 39.3±15.6 pg/ml and 51.7±34.9 pg/ml respectively. All the above measured parameters were significantly different among the three stages of disease, with higher values with advancing disease stage (p<0.001 in all cases). Furthermore in the entire group of patients with MM serum levels of IL-6, IL-8 and MIP-1α were significantly higher in patients in comparison to controls (p<0.001, p<0.002, p<0.001 respectively). A positive statistical correlation was found between IL-6 and IL-1β (r=0.462 p<0.002), IL-6 and IL-8 (r=0.358 p<0.01), IL-6 and MIP-1α (r=0.380 p<0.001). Similarly IL-8 and MIP-1α were positively correlated with factors of disease activity such as B2M (r=0.502 p<0.001, r=0.413 p<0.005 respectively) and LDH (r=0.415 p<0.006, r=0.475 p<0.001 respectively). PCNA immunostaining was identified in the nuclei of the cells in all the cases of the disease. Concerning the three stages of the disease, the proliferation index as assessed by PCNA immunostaining was evaluated in stage 1 with a mean value 6.6±5.2% (range 1-15%), in stage 2 24.0±12.4% (range 5-50%) and in stage III 41.4±29.0% (range 10-98%). In the entire group of MM PCNA expression was higher with advancing disease stage (p<0.001). Furthermore PCNA expression correlated significantly with parameters of disease activity such as B2M and LDH (r=0.406 p<0.006, r=0.581 p<0.0001 respectively). Similarly the expression of myelomatous positive PCNA protein correlated with the measured cytokines IL-6, IL-1β, IL-8 and MIP-1α (r=0.520 p<0.0001, r=0.545 p<0.0001, r=0.320 p<0.03 and r=0.358 p<0.01, respectively). Conclusions. Our results showed that the proliferative activity, as assessed with PCNA, was increased in parallel with disease stage. The positive correlation between PCNA and other measured mediators, such as IL-6, IL-1β, IL-8 and MIP-1α, supports the involvement of these factors in the biology of myeloma cell growth and can be used as markers of disease activity and as possible therapeutic targets.

0853
NEPHELOMETRIC MEASUREMENTS OF FLC κ AND FLC λ FOR MONITORING LIGHT CHAIN MULTIPLE MYELOMA PATIENTS
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Introduction. Serum free light chain (sFLC) measurements are important in multiple myeloma (MM), have replaced urine electrophoresis in diagnostic algorithms and are required to characterise a complete response. However, with the exception of oligosecretory disease, their use is not recommended in current guidelines for routine monitoring. Aim. To investigate the use of sFLC measurements for monitoring light chain MM (LCMM) patients. Methods. sFLC κ and sFLC λ levels were measured nephelometrically on serial samples from 25 LCMM patients (14 FLCK and 11 FLClx); specifically at presentation, after cycles 2 and 4 of therapy and post autologous stem cell transplantation (ASCT). The results were compared with historic urine electrophoresis (UPE) plus urine and serum immunofixation electrophoresis (sIFE) and sIFE; to identify residual disease sIFE was performed on all patients after 2 cycles of therapy. Results. At presentation all patients had a detectable band on UPE and measurable disease using UPE (>2000mg/L). 22/25 patients had a detectable monoclonal band by sIFE, with 1/25 patients having a monoclonal band quantifiable by SPE (11.5g/L). In the FLCK subgroup of patients, all 14/14 patients had increased FLCK levels in the serum. The median FLCK was 3740 mg/L (range, 689-13100 mg/L). Additionally, all 14 FLCK patients had abnormally increased FLCK/κα ratios, with a median value of 552 (range, 42-2400). In the FLClx subgroup, all 11/11 patients had both increased FLClx levels and abnormally decreased FLCK/κα ratios in the serum, with the median value of 3000 mg/L (range, 875-22000 mg/L) and a median ratio of 0.001 (0.0001-0.02). Table 1 shows the patient responses as...
sessed using UPE/UFE or sFLC measurements alone; there were no patients with positive UFE results and normal FLC ratios. After 2 cycles of therapy a normal FLC ratio (18/20) correlated well to sIFE (17/20) results in the identification of residual disease (Pearson’s r=0.7, p<0.001). The median involved FLC (iFLC) level in these patients was 152 mg/L (range, 52–202 mg/L). UFE (13/20) under-estimated the number of patients with residual disease and correlated less well to sIFE (Pearson’s r=0.5, p=0.08). Post ASCT, sIFE and sIFE indicated CR in 15 patients, however, sFLC measurements detected residual disease in 5 patients (median iFLC level of 25 mg/L (range, 15–25.55 mg/L)). Discussion. sFLC measurements correlated well to sIFE measurements in the detection of residual disease. There were no instances when residual disease was detectable by UFE or UFE but not detectable by FLC measurements. In conclusion, FLC measurements could replace UFE and UFE to effectively monitor LCMM patients.

### 0854

**MONITORING SERUM LEVELS ELR+ CXC CHEMOKINES AND RELATIONSHIP BETWEEN ANGIOGENESIS DENSITY AND ANGIogenic Growth FACTORS IN MULTIPLE MYELOMA**

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**Background.** The ELR+ CXC chemokines are important mediators of angiogenesis, related to their angiogenic properties. Angiogenesis appears to be a prominent feature in the progression of multiple myeloma (MM). CXC chemokines have four highly conserved cysteine amino acid residues, with the first two cysteine molecules separated by a single amino acid. The angiogenic potential of this group is determined by the presence of three amino acid residues (Glu-Leu-Arg: the ELR motif) preceding the first cysteine amino acid, in the NH2 terminus. **Aims.** The purpose of this study was to determine serum concentrations of angiogenesis-related chemokines ELR+ motif, such as interleukine-8 (IL-8), epithelial neutrophil activating protein-78 (ENA-78) and growth-related gene alpha (GRO-α), as well as the bone marrow microvascular density (MVD) in patients with MM at diagnosis and after treatment, in plateau phase. We also evaluated the relationship among them with other known growth factors involved in angiogenesis, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and tumor necrosis factor-α (TNF-α). **Methods.** Serum levels of the above chemokines were determined in 68 newly diagnosed MM patients, in 28 in plateau phase and in 20 healthy controls. Serum measurements of IL-8, ENA-78, GRO-α, VEGF, HGF and TNF-α were performed with a commercially available kit for ELISA. Bone marrow biopsies were performed before and after treatment, in plateau phase, in order to determine MVD by staining vessels with anti-CD31. **Results.** Serum concentrations of IL-8, ENA-78, GRO-α and TNF-α were significantly higher in the group of MM patients (44.5±25.3pg/ml, 765±572.1pg/ml, 186±129.1pg/ml and 4.2±2.8pg/ml respectively) in comparison to control group (27.2±6.3pg/ml, 355.1±268.6pg/ml, 112.5±76.1pg/ml and 1.3±0.8pg/ml) (p<0.0004, p<0.002, p<0.01 and p<0.0001 respectively). We also found that untreated patients had higher levels of IL-8, ENA-78, GRO-α and TNF-α than treated patients, but statistical significant difference was found only for IL-8 (48.36±30.93pg/ml vs. 35.05±19.77pg/ml, p<0.001). Furthermore IL-8, GRO-α, TNF-α, HGF and VEGF were significantly higher in increasing disease stage (p<0.001 in all cases). ENA-78 serum levels were higher in stage III than in stage I and II, but without statistical significance. Additionally we correlated each proinflammatory cytokine with well known angiogenic factors such as HGF, VEGF and TNF-α. A positive correlation was found between serum HGF and IL-8 and GRO-α (r=0.316 p<0.01, r=0.297 p<0.01 respectively). Similarly serum VEGF correlated positively with ENA-78 and GRO-α (r=0.314 p<0.04 respectively) while a trend to correlate was found between TNF-α and IL-8 (r=0.242 p<0.056). In the pretreatment group of patients a positive correlation between bone marrow MVD and serum levels of GRO-α was found (r=0.304 p<0.01) while MVD did not correlate with IL-8 and ENA-78. There was a difference in survival times between patients with levels higher than median versus low IL-8, ENA-78 and GRO-α levels, but the differences could not reach statistical significance in either case. **Conclusions.** These findings support the hypothesis that ELR+ motif CXC chemokines, such as IL-8, ENA-78 and GRO-α correlate with angiogenic growth factors and may play a role in the progression of MM. Further studies are needed to determine their prognostic and predictive significance.
IMMUNE-RELATED CONDITIONS INCREASE THE RISK OF MULTIPLE MYELOMA AND MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: RESULTS FROM A POPULATION-BASED STUDY
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Background. The etiology of multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS) is largely unknown. There is evidence for genetic factors in the etiology of MM and MGUS. Several study groups have shown that immune dysregulation plays a major role in lymphomagenesis. Much less is known regarding immune dysregulation in MM and MGUS. There is evidence for genetic factors in the etiology of MM and MGUS. gammopathy of undetermined significance (MGUS) is largely unknown.

Aims. The aim of this study was to evaluate the expression of CCL3 by MM cells in bone marrow biopsies of WM patients to test the hypothesis of production of CCL3 by WM cells. Methods. We evaluated bone marrow biopsies from 67 patients with WM (90±28 years; median: 72 years; range: 39-85 years) who were diagnosed, treated, and followed-up in a single center (Department of Clinical Therapeutics, University of Athens, Greece) between 1999 and 2009. Forty-six patients had newly diagnosed WM (4 with asymptomatic disease), while 21 patients had active disease after treatment. Bone marrow biopsy sections were immunohistochemically tested for the expression of CCL3 (using an anti-CCL3 monoclonal antibody by Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD20, CD79alpha, CD138, MUM-1, as well as for mu, alpha, gamma heavy and kappa and lambda light immunoglobulin chains. The immunoreactivity of CCL3 was examined on the basis of positive lymphoplasmacytic and/or plasma cells with a cut-off value of >20% positive cells. Results. In all MM cases, either at diagnosis or at relapse, the whole number of the neoplastic cells, including CD20(+)/CD138(+)/MUM-1(-) small B-lymphocytes, plasma-cyroid lymphocytes and rare immunoblasts as well as CD20(-)/CD138(+)/MUM-1(+) plasma cells revealed strong cytoplasmic positivity for CCL3. Due to such strong positivity for CCL3, no definite correlation could be found between CCL3 expression by the neoplastic cells and disease features were able to be established. Regarding the putative role of CCL3 overproduction in WM, only hypotheses can be made. In chronic lymphocytic leukemia (CLL), an abnormally high number of infiltrating CD68+ monocytes/macrophages has been described in the CLL-involved areas of trephine biopsies from CLL/CCL4-producing CLL cells. Thus, we performed a CD68 (PGM-1) staining in the trephine biopsies of our symptomatic WM patients and found a high number of CD68+ cells in the infiltrating areas. These cells may contribute to the WM cell survival in a similar way observed in CLL. CCL3 is also a chemoattracting factor for monocytes/macrophages, and it is well known that the mast cells support lymphoplasmacytic cell growth in WM. In all our cases, there was a high number of mast cells in the infiltrating areas, supporting a possible role of CCL3 in this accumulation. Summary/Conclusions. CCL3 is overexpressed by WM cells. This result supports a possible role of CCL3 in WM biology through interactions of the malignant clone with the bone marrow microenvironment and reveals CCL3 as a potential target for developing novel drugs against WM.
1.48 vs 2.62 ± 0.43 g/L, P < 0.001). The highest fibrinogen was found in LCD subgroup. Fibrin clots in MM patients compared with those obtained from controls had lower clot permeability (5.19 ± 1.28 vs 6.89 ± 0.77 10⁻⁹ cm², respectively, P < 0.001), prolonged clot lysis time (12.5 ± 2.2 vs 8.06 ± 0.94 min, P < 0.0001), and lower baseline maximum clot absorbancy at 405 nm (0.56 ± 0.1 vs 0.79 ± 0.06, respectively, P < 0.001). Baseline maximum velocity of D-dimer release from plasma clots subjected to tissue plasminogen activator was lower in the MM group than in controls (0.065 ± 0.006 vs 0.077 ± 0.006 mg/L/min, P < 0.001), while the initial maximum D-dimer level was higher in MM patients (4.76 ± 0.62 vs 3.48 ± 0.27 mg/L, P < 0.0001). MM patients with renal insufficiency (n = 16) had significantly lower clot permeability than the remainder (n = 25, P < 0.015). Comparison of fibrin clot obtained from patients after treatment with thalidomide with that prior the treatment did not show changes in fibrinogen concentrations (3.9 ± 1.2 vs 4.03 ± 1.48 g/L, P < 0.2), in clot permeability (5.49 ± 1.3 vs 5.19 ± 1.28 10⁻⁹ cm², P < 0.4), or in clot lysis time (12.3 ± 1.78 vs 12.25 ± 2.12 min, P < 0.7) but maximum clot absorbancy (0.72 ± 0.1 vs 0.56 ± 0.1, P < 0.005) and the rate of D-dimer release from clots (0.073 ± 0.002 vs 0.066 ± 0.006 mg/L/min, P < 0.009) was higher. Out of 38 MM patients treated with thalidomide 6 subjects developed venous or arterial thrombosis and they had lower clot permeability compared to the remaining individuals (P < 0.05); other fibrin clot variables were similar in both subgroups. Conclusion. Substantial prothrombotic alterations of fibrin clot properties, including markedly impaired fibrinolysis occur in patients with MM and thalidomide-containing regimens further modify clot phenotype, which seems to contribute in thrombotic complications.

**0859**

**IMPACT OF NOVEL M-COMPONENT BASED BIOMARKERS ON TO PROGRESSION FREE SURVIVAL AFTER TREATMENT IN MULTIPLE MYELOMA PATIENTS**

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**Background.** The depth of response has been associated with longer Progression Free Survival (PFS) in Multiple Myeloma (MM). Serum free light chains (sFLC) and ratio of sFLCR, are used for the evaluation of sCR. They constituted the background for developing specific antibodies that bind the junction of the heavy and light chains on individual immunoglobulin isoforms (Helyvite®), making possible to quantify the IgGκ, IgGλ, IgAκ, IgAλ, and their ratios IgGκ/IgGλ, IgAκ/IgAλ, and IgGκ/IgAκ and IgGλ/IgAλ (HLCRs) separately. Aim: To investigate the importance of sFLCR and HLCR in correlation on PFS. Parameters and Methods. 51 intact immunoglobulin MM patients were studied from diagnosis to last follow up. All patients were symptomatic. Their sera samples (n = 312) were analyzed for sFLC by immunoassay (Freelite®) and for IgGκ, IgGλ, IgAκ, IgAλ, with Helyvite® antibody, nephelometrically. Serum FLCR and HLCR values above the 95%-ile of normal individuals were considered as abnormal. File data were reviewed. Results. Median lines of therapy were 2 (range 1-11). Median follow up was 28 months (4-135). Retreatment was initiated in all patients according to standard criteria. HLCR was abnormal in all patients at diagnosis. As expected the quality of response correlated to FFS and patients in sCR, CR and nCR had a longer FFS than the others. Both sFLCR and HLCRs normalization were strong parameter of increased PFS after treatment at any line (p = 0.29 and p = 0.16, respectively). Conclusion. Both sFLCR and HLCRs normalization reflect prolonged responses.

**0860**

**HLA-DRB1*13 AND *15 ARE ASSOCIATED WITH RESPONSE TO THALIDOMIDE IN PATIENTS WITH MULTIPLE MYELOMA**

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**Background.** Thalidomide (T) has been approved for treatment of multiple myeloma. There is no generally accepted predictor for response. Aim: To study the impact of HLA antigens on response to T. Methods. Patients (n = 46) who received T either as monotherapy (n = 36) or combination therapy with dexamethasone (n = 10) were retrospectively analyzed. Patient characteristics were: median age 50 (31-73), male/female 31/15, IgG/IgA/IgM/light chain 33/6/1/6 and ISS-I/II/III 21/10/11. Numbers of patients given T for induction/consolidation/relapse were 2/18/26. Patients were given T at doses 50-400 mg for a median duration of 10 (2-72) months. Thalidomide was stopped whenever disease progression or side effects grade ≥ 3 occurred. Response to T was classified as responders ± partial remission (PR) or non-responders (disease progression on T). Results. 16 patients had a response, 18 patients had disease progression and 12 patients had stable disease on T. Parameters which may have contributed to response to T were compared between responders and non-responders (Table 1-2). There were no statistically significant difference between the responders and non-responders in terms of age, sex, paraprotein, prognostic subgroup or the number of previous chemotherapy cycles. The only significant differences were the number of previous chemotherapy lines and the HLA frequencies. Summary/Conclusions. In a recent study (Beksar et al. 2008), it was demonstrated that HLA-DRB1*15, HLA-DRB1*13 and HLA-DRB1*11 were observed 7.2%, 18.2% and 21.1% in MM population, respectively. In this study, the percentage of response to T in HLA-DRB1*11 were observed 7.2%, 18.2% and 21.1% in MM population, respectively. In this study, the percentage of response to T in HLA-DRB1*11 positive and HLA-DRB1*13 positive patients were 55.5% and 66.6%, respectively. There were 4 patients who were HLA-DRB1*15/13 and all of them responded to T. On the other hand, HLA-DRB1*11 was associated with refractoriness to T (11% response, p = 0.01). Host related factors such as HLA may have impact on response to T similar to that observed in aplastic anemia-immunosuppression-HLA-DRB1*15.

**0861**

**CORRELATION OF SERUM FREE LIGHT CHAIN QUANTIFICATION WITH SERUM AND URINE IMMUNOFIXATION IN MONOCLONAL GAMMOPATHIES**

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**Objectives.** Standard methods for detection and monitoring of monoclonal proteins include electrophoresis and immunofixation (IFE) of...
serum and urine samples. Recently, automated quantitative immunoassay for serum immunoglobulin free light chains (FLCs) has become available for clinical use. The aim of this study was to assess the efficacy of serum FLC assay in detection and monitoring of monoclonal proteins in different variants of monoclonal gammopathy. Material and Methods. Diagnostic value of serum FLC κ/λ ratios was compared with serum and urine protein electrophoresis and IFE in 106 multiple myeloma patients, solitary plasmacytoma patients and 8 patients with monoclonal gammopathy of undetermined significance (MGUS). FLCs were measured using Siemens BNII nephelometer with the FreeliteTM Kit (Binding Site). Results. Of 37 light chain (LC) myeloma patients, 21 were positive for κ LC and 16 for λ LC. All of these patients had monoclonal LC detected in urine (IFE) and LC also detected in serum. Although 27 patients had M spike in urine, only 9 had M spike in serum. All patients in κ subgroup had an increased κ FLCs in serum and increased FLC ratio. In λ group all patients had both increased λ FLCs and decreased FLC ratio in serum. In 5 of 8 patients with nonsecretory myeloma and negative serum and urine IFE, the FLCs were increased. In all 58 myeloma patients with intact immunoglobulin (G 26, A 8, M 1, D 2, E 1) assessed at diagnosis or in disease progression, the FLC ratio was abnormal; in 3 patients in CR after bortezomib therapy the FLC ratio was normal and in 2 of 3 patients with PR the FLC ratio was abnormal. In 13 of 29 patients treated with autologous stem cell transplant the normal FLC ratio documented stringent complete responses. Discrepancy between IFE and FLC results was found in 5 patients. Abnormal FLC ratio in 3 patients was ahead of the disease progression that was revealed in IFE and histology. In contrast, in one case, the appearance of M-protein in IFE and increased plasma cell rate in bone marrow was observed earlier in relation to FLC level which remained normal. Immunoglobulins with solitary extramedullary plasmacytoma the FLC ratio was normal. Three of 8 MGUS patients with follow-up from 10 to 26 years had abnormal the FLC ratio. Multiple myeloma progression developed in one patient with normal FLC ratio. Conclusion. Serum FLC assay allows improved detection rates of light chain multiple myeloma and nonsecretory multiple myeloma. This test enables also monitoring of these diseases, and allows more precise definition of remission status.
those reported in other countries. The elderly population has higher serum from light chains concentrations due to worsening renal function in this group of people.

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0864

**MYELOMA CELL CONTAMINATION OF PERIPHERAL BLOOD STEM CELL HARVESTS ESTIMATED BY MULTIPARAMETRIC FLOW CYTOMETRY: POTENTIAL CORRELATION WITH COMPLETE REMISSION RATE AND DURATION**


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Background. High-dose therapy and autologous stem cell transplantation (ASCT) remains an integral component of the myeloma treatment algorithm for patients considered eligible for the procedure. Recent studies suggest that the presence of malignant plasma cells in the peripheral blood stem cell harvest (PBSCH) may correlate with the clinical outcome after high dose chemotherapy followed by ASCT. Objectives. To estimate the presence and the proportion of plasma cells (PC) in PBSCHs in correlation with clinical data. Methods. PBSCHs from 30 myeloma patients (21 men and 9 women, n = 30) who underwent ASCT were studied by 6-color flow cytometry (FCM) using the following combination of fluorochrome-conjugated antibodies, CD38/CD56/CD45/CD117/CD138/CD19. Response to therapy was assessed at the time of mobilization, at day +100 and at the end of the study after 14 months median period of follow up. The 2006 International Myeloma Working Group criteria were applied as follows: complete response (CR) (n=8), very good partial response (VGPR) (n=8), and partial response (PR) (n=14). Mobilization regimens included G-CSF (n=30), Cyclophosphamide (n=3), Plerixafor (n=2). The Chi-square test was used to determine the p-value comparing the variables. Pearson correlation coefficient was applied to establish a correlation between variables and the Mann-Whitney U test for continuous variables. Results. Plasma cells bearing CD138+CD38+CD45+CD19+CD117-CD56- immunophenotype were identified in 29/30 cases. The mean PC percentage by FCM was 0.029% (0-0.27%). Plasma cell contamination compared to the VGPR/PR group (mean 0.0046% vs. 0.03%, p=0.006). Patients with low or undetectable contamination in this group of people.

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0865

**PATTERNS AND EFFECTIVENESS OF BORTEZOMIB USE IN ELDERLY PATIENTS: THE VESUVE COHORT**


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Background. Bortezomib (BTZ) represents an important progress in the treatment of multiple myeloma (MM). BTZ combined with other agents is becoming a standard of care particularly in elderly patients not eligible for autologous stem cell transplantation. To date, no evaluation of BTZ in a real-life setting has been conducted in France. Aims. To describe and compare patterns and effectiveness of BTZ use in two age groups: ≤75 years (younger) vs >75 years (older). Methods. VESUVE is a national cohort conducted in 60 French centres that included patients initiating BTZ from May 2004 to April 2006 using nonimbursement hospital pharmacy dispensations. Patients treated for MM were followed for 2 years. Data regarding treatment modalities, response and survival outcomes (overall survival, OS and progression-free survival, PFS) were collected through medical files. BTZ cycles were categorized as standard or not according to market authorisation (dose, injection and cycle rhythm). Response was assessed by an independent committee according to adapted International Myeloma Working Group criteria. Results. Among the 793 patients included, 82.3% were aged ≤75 years and 17.7% >75 years. Concomitant use of other agents was more frequent in youngers (73.5% vs 68.0%; p=0.04) especially regarding conventional chemotherapy (16.7% vs 1.1%; p<0.01). Mean ±SD number of BTZ cycles did not differ between groups (5.0±3.5 vs 4.6±3.0; p=0.15) but mean BTZ cumulative dose per cycle was 7.9±1.6 mg in younger vs 7.1±1.9 mg in older (p<0.01). The proportion of patients with at least one non-standard cycle was 62.0% in younger vs 77.9% in older (p=0.02). BTZ was withdrawn for safety reasons in 20.1% in younger vs 26.4% in older (p=0.10). Among the 588 evaluable patients for response, the overall best response rate was 57.1% in younger vs 64.1% in older (p=0.19). The 2-year OS rate was 44.2% (95% CI 40.2-48.0; median 20.4 months) in younger vs 36.5% (23.2-44.4; 14.1 months) in older (p=0.02). The 2-year PFS rate was 11.6% (9.3-14.5; 7.3 months) in younger vs 13.8% (8.6-20.3; 6.5 months) in older (p=0.69). Conclusions. Despite differences regarding use, these results show that BTZ leads to very similar survival outcomes in patients over 75 years of age as compared to younger patients.

0866

**CONSOLIDATION/MAINTENANCE THERAPY AFTER INTENSIVE INDUCTION IMPROVES QUALITY OF RESPONSE AND OUTCOME IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA (R-RMM)**


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Background. There are some evidences that consolidation/maintenance therapy improves quality of response and remission duration in newly diagnosed MM. However, the impact of these strategies in relapsed/refractory MM (r-MM) are still unknown. Aims. We assess the impact of consolidation/maintenance (C-M) therapy on quality of response and outcome, performing a post-hoc analysis of a prospective phase II study exploring an intensive combination as induction followed by consolidation and maintenance therapies in r-MM. Methods. Patients

Myeloma and other monoclonal gammapathies - Clinical 1
received 6 28-day cycles of oral thalidomide 100 mg/day continuously, oral dexamethasone 20 mg/day on day 1-2, 4-5, 8-9, 11-12, pLD 30 mg/m² iv on day 4 and Velcade 1.3 mg/m² iv on day 1, 4, 8, 11 plus dexamethasone 20 mg on day 1-2, 4-5, 8-9 (3 courses) and thalidomide 100 mg/day continuously plus dexamethasone 20 mg on day 1-4 (3 courses). Maintenance therapy included thalidomide 100 mg/day until relapse or intolerable toxicities. Due to an excess of peripheral neuropathy, protocol was amended as follows: Velcade 1.3 mg/m² on day 1, 4, 11 and thalidomide 50 mg/day in all therapeutic phases. Response, time to progression (TTP), progression free survival (PFS) and overall survival (OS) were assessed according to International Myeloma Working Group (IMWG). To assess the role of C-M therapy we compared TTP and OS of patients who were able to continue planned therapy with those of patients not receiving C-M because of toxicity by a 6-months landmark analysis. Results. Median age of 46 patients was 63.5 years (range 31-85 years) and the median number of prior regimens was 1 (range 1-4). Fifty to percent had undergone autologous stem cell transplantation, 59% thalidomide, 17% bortezomib and 35% were refractory to the last regimen. After induction 13% of patients achieved sCR, 21.5% CR, 32.5% VGPR and 8.5% PR. Additional 4 patients had SD while 7 progressed. Out of 46 patients undergone induction, 26 (56.5%) received consolidation therapy since 10 patients progressed or died during induction, and 10 developed severe toxicities requiring therapy interruption. Five (25%) among the 20 patients without a prior sCR further improved response. Therefore, the best response after induction and consolidation was 17.5% sCR, 19.5% CR, 54.5% VGPR and 4.5% PR. The 20 patients receiving thalidomide maintenance therapy had no improvement in response. Globally, median TTP and PFS were 18.5 and 17.5 months, respectively while median survival was 40 months. Patients achieving sCR had a significantly better 3-year TTP if compared with those obtaining CR (86% vs 46%; p = 0.005) or VGPR-PR (86% vs 11%; p = 0.005). Patients who completed the protocol had a significantly longer TTP (NR vs 7 months; p = 0.001) and OS (NR vs 28 months; p = 0.035) compared with those who did not. Conclusions. This retrospective analysis suggests that, in r-MM, the possibility of receiving intensive and continuous therapy containing bortezomib and thalidomide allowed to improve of response that translates into a significantly better outcome.

**Table 1.**

## Baseline Characteristics and Efficacy of Bortezomib-Based Therapy in Relapsed/Refractory Multiple Myeloma: Results After Complete Enrollment from an Electronic Bortezomib Observational Study (eVOBS)

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**Background.** The international, non-interventional electronic bortezomib observational study (eVOBS) is ongoing to prospectively evaluate the efficacy and clinical outcomes associated with bortezomib-based therapies for primarily relapsed/refractory multiple myeloma (MM) in the ‘real-world’ clinical practice setting. Aims. With study enrollment completed, we report patient demographics, baseline disease characteristics, and efficacy data for the entire population included in eVOBS. Methods. Adult patients scheduled to begin bortezomib-based therapy for the treatment of predominantly relapsed/refractory MM were eligible for enrollment into the study. Patients were enrolled at clinical practices in Belgium, Brazil, Canada, France, Greece, Russia, Spain, Sweden and Turkey, and provided written informed consent. Treatment history was retrospectively documented for 1 year prior to initiation of bortezomib-based therapy. Prospective observational data were collected for up to 3 years after initiation of bortezomib-based therapy to document efficacy data. All administered bortezomib doses and concomitant treatments were permitted, except for investigational therapies. MM disease stage was assessed at the time of initiation of bortezomib-based therapy using Durie-Salmon (DS) or International Staging System (ISS) criteria. Responses were investigator-assessed and based on European Group for Blood and Marrow Transplantation (EBMT), Southwest Oncology Group (SWOG), or M-protein criteria. Response data were censored at the start of subsequent therapy, and for deaths. Results. 1560 patients were enrolled between October 2006 and December 2010. Sociodemographic, chronic co-morbidities, and patient characteristics were recorded at baseline (Table). The majority of patients (97%) included in this study received bortezomib-based therapy for relapsed/refractory MM; 3% of patients received bortezomib as their first-line therapy for MM. The most common prior MM treatments documented within 1 year of starting bortezomib-based therapy were melphalan-prednisone combinations (20%), thalidomide-dexamethasone (9%), vincristine-doxorubicin-dexamethasone ± cyclophosphamide (9%) and thalidomide monotherapy (6%); 5% of patients had an autograft and 41% had no prior MM treatment in the previous year. The majority of patients received bortezomib in combination with other agents including dexamethasone (44%), prednisone (9%, mostly with melphalan), thalidomide (4%, mostly with dexamethasone), and lenalidomide (2%); 30% of patients received bortezomib monotherapy. Most (84%) patients received bortezomib at an initial dose of 1.3 mg/m². At the data cut-off of 08 January 2011, and after a median follow-up of 15.5 months, 26.9%, 16.7%, 32.5%, and 8.4% of patients achieved a complete response (CR), near-CR (nCR), partial response (PR), and minimal response (MR), respectively. In responding patients, the median time to CR, nCR, PR, and MR was 3.9, 3.2, 1.9, and 1.6 months, respectively. Conclusions. The eVOBS database contains valuable information about the efficacy of bortezomib-based therapies for relapsed/refractory MM in ‘real-world’ clinical practice. These results provide a useful comparator for data collected in the highly controlled clinical trial setting. Patients continue to be followed for long-term outcomes.

**Table 2.**

## Better Treatment Response is Associated with Lenalidomide 25 mg Once-Daily vs. 25 mg Every-Other-Day in Relapsed/Refractory Multiple Myeloma

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**Background.** This retrospective analysis suggests that, in r-MM, the possibility of receiving intensive and continuous therapy containing bortezomib and thalidomide allowed to improve of response that translates into a significantly better outcome.
strated significant improvements in time to progression (TTP) and overall survival (OS) in patients with relapsed/refractory multiple myeloma who have received a 1 prior therapy. A pooled analysis (Dimopoulos et al. Leukemia. 2009;23:2147-2152) of two large multicenter phase III trials (MM-009 and MM-010) assessed long-term outcomes (median follow-up 48 months in surviving patients) of 25 mg once-daily lenalidomide plus dexamethasone vs. placebo plus 40 mg dexamethasone. This analysis demonstrated significant response and survival benefit with the lenalidomide plus dexamethasone combination (overall response rate (ORR) of 60.6% in patients receiving lenalidomide plus dexamethasone compared with 21.9% in patients receiving dexamethasone plus placebo). Median OS for the combination was 38.0 months with the combination and 31.6 months for patients receiving dexamethasone plus placebo. Aims. To evaluate treatment response (CR, VGPR, PR, MR, SD and PD), OS, and safety with lenalidomide 25 mg given once-daily or every-other-day for 21 days of a 28 day cycle in patients with relapsed/refractory (47%) multiple myeloma. Methods. In this clinical practice chart review, 169 evaluable patients, 58% male, median age 64.3 years, received 25 mg once-daily (n=140) or every-other-day (n=29) oral lenalidomide-based regimens (57% with glucocorticoids (87% dexamethasone),160 or 320 mg per cycle); 43% with glucocorticoids plus conventional cytarabine agents. Patients treated with lenalidomide monotherapy were excluded from this analysis. Patients in both groups received a median of 3 previous lines of therapy, 89% and 86% with ≥2 therapies, in the once-daily and every-other-day lenalidomide groups, respectively. Results. VGPR was 7.9% and 0%, PR was 30.3% and 10.0%, and MR was 67.2% and 80.0% with lenalidomide 25 mg once-daily and every-other-day, respectively. Response rate and median time to follow-up of 8.4 months (range 0.3 - 26.2), ORR (≥PR) was 38.2% versus 10.0%. Based on the definition of response (60 days after end of treatment), 59 and 10 patients were evaluated. Median OS from the start of therapy was 16.7 and 10.3 months in the lenalidomide 25 mg once-daily and every-other-day groups (evaluated in 140 and 29 patients, respectively). Lenalidomide once daily did not result in significantly higher incidence of hematologic adverse events. Grade 3/4 adverse events included neuropathy (8.6%, 3.4%), thrombosis (8.6%, 3.4%), infection (14.9%, 3.6%), thrombocytopenia (12.4%, 3.4%), neutropenia (34.1%, 27.6%), and anemia (14.8%, 6.9%) in the once-daily and every-other-day lenalidomide groups. There was no significant difference between the two treatment groups in grades 1-5 thrombocytopenia or neutropenia. One patient treated with 25 mg once-daily lenalidomide experienced grade 5 thrombocytopenia and one patient experienced grade 5 infection. Conclusions. Lenalidomide is active and generally well-tolerated in relapsed/refractory multiple myeloma and indicate that every-other-day dosing compromises response with no significant difference in tolerability.

0870
RENAL FUNCTION IMPROVEMENT EVALUATED BY CYSTATIN-C SERUM LEVELS IN PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS TREATED WITH MEL-DEX ASSOCIATION
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Background. Primary systemic AL amyloidosis is a clonal plasma cell disorder in which the N-terminal fragments of monoclonal light chains form fibrils that accumulate in various tissues ultimately leading to organ dysfunction and death. Renal injury is a very frequent feature in AL amyloidosis patients. Serum cystatin-C and free light chain (s-FLC) κ and λ levels were investigated to evaluate clinical severity degree of renal impairment in AL amyloidosis, also in terms of prognosis. Cystatin-C is a non-glycosylated protein with low molecular weight (13kDa) produced at a constant rate in all nucleated cells. It is freely filtered, reabsorbed for 99% in proximal tubule and not secreted so it has higher sensitivity than creatinine determination for detecting initial GFR reduction. This peculiarity may be useful for evaluation of kidney and heart dysfunction for patients with systemic diseases. Aims and Methods. We evaluated serum cystatin-C and s-FLC levels in 7 patients (median age 63.1 yrs) with recent diagnosis of systemic AL amyloidosis admitted to our Unit. Ten age-matched healthy control subjects were selected. According to age and disease risk stratification six out seven patients were treated with upfront oral MelDex association (Melphalan 9 mg/sm, Dexamethasone 20mg day 1-4 q28). One subject started first line therapy with BorDex association (Bortezomib 1.3 mg/sm, Dexamethasone 20 mg day 1, 4,8,11 q21). Three samples of peripheral blood were performed (treatment day 1, day 8 and at con-
Conclusion of the first cycle of therapy). The blood was separated into 4 patients with persistent high serum levels of cystatin-C at the end of cycle, 3 had poor outcome (2 deaths with heart and renal failure respectively, 1 alive with end stage renal disease in haemodialysis). Conclusions. On the basis of these results we could consider cystatin-C a useful biomarker for renal function assessment with prognostic value according to treatment in patients with systemic AL amyloidosis.

**0871**

**HIGH RESPONSE RATE IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH A COMBINATION OF CYCLOPHOSPHAMIDE, LIPOSOMAL DOXORUBICIN, DEXAMETHASONE AND BORTEZOMIB**

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**Background.** Autologous stem cell transplantation (ASCT) and the use of new agents have significantly improved the outcome of multiple myeloma patients. The achievement of complete response after induction treatment is prerequisite for long term overall survival. Although ASCT is still considered standard of care, its necessity in first relapse remains challenging in the era of the new agents. Aim. The aim of our study was to evaluate the efficacy and toxicity of an intensive treatment protocol in newly diagnosed patients with multiple myeloma. Methods. 26 patients (21 male, 5 female) of median age 60 years (48-75) were treated after informed consent with a combination of cyclophosphamide 750mg/m2 d1, liposomal doxorubicin 40mg/m2 d1, dexamethasone 40mg d 1-4 and bortezomib 1.8mg/m2 d1,4,8,11 every 28 days. All patients also received zoledronic acid 4 mg every 28 days and erythropoietin to maintain hemoglobin > 10.5 gr/dl. The median follow up was 17 months (3-40). The ISS stage was: I 7 (27%), II 15 (58%) and III 13 patients. The monoclonal globin type was IgG in 12 patients, IgA in 9, IgD in one, IgE in one and free light chain in 3. Renal impairment (Cr>2) was present in 5 patients and hypercalcaemia in 7 patients. Radiotherapy was applied in 7 patients. Results. All patients (n=26) who received at least 2 cycles of therapy were evaluated. The median number of cycles was 6 (range 2-26). Thirteen patients (50%) had complete response (6 nCR, 7 CR IF-), 7 (27%) patients had very good partial response (VGPR), 3 (11.5%) had partial response (PR) while 3 (11.5%) patients didn't respond or had progressive disease. Three patients in CR had a sufficient collection of CD34+ cells but only one finally underwent ASCT. The toxicity of the treatment was acceptable. Haematological toxicity: neutropenia grade III 2/26, grade II 5/26, grade IV 1/26, thrombocytopenia grade II 7/26, grade III 2/26. In terms of non-haematological toxicity, one patient had deep vein thrombosis and more than half of the patients experienced gastrointestinal disturbances. Two patients were hospitalized with neutropenic fever. Maintenance treatment with thalidomide 50mg/d or lenalidomide 10-15 mg/d 1-21/mo after the achievement of CR or VGPR was introduced to 10 patients for at least 6 months. The progression free survival for the patients who achieved CR or VGPR was introduced to 10 patients for at least 6 months. The progression free survival for the patients who achieved CR or VGPR was 14,5 months. The probability of relapse is 85% at 36 months. Four patients died, one in CR and three in relapse due to infections. The probability of survival at 36 months is 68%. None of the analyzed factors in mono- and polyparametric analysis were significant for probability of relapse and survival. Conclusions. The above protocol was efficient in terms of response rate. It was safe with acceptable and manageable toxicity. Addition of bortezomib in 1st line treatment significantly improves the response rate. Larger number of patients and longer follow up is needed to define if our approach may lead to improved progression free and overall survival, without the necessity of ASCT in first CR.

**O872**

**ANALYSIS OF THE INCIDENCE, CLINICAL AND BIOLOGICAL CHARACTERISTICS OF IGM MONOClonAL GAMMAPATHY DETECTED IN a URBAN POPULATION STUDY**


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**Background.** Several studies have examined the prevalence of Monoclonal Gammapathy of Undetermined Significance (MUGS) and its characteristics. However, few studies have focused on IgM Monoclonal Gammapathies (MG), especially IgM MUGS, entities with different pathophysiological significance and follow-up, opposite to non-IgM MUGS. Aims. To analyze the incidence, clinical and biological characteristics of the IgM MG detected in a urban population study. Material and Methods. Prospective cohort study in the urban population over the age of 50 years old in Segovia (Spain). The serum monoclonal component was detected by electrophoresis and confirmed by immunofixation. The selection of the participants was done through Health Centers or personal letter. Results. At the first 26 months, it has been consented by 16161 people of whom 6681 agreed to take part in the above mentioned study. 147 MG was detected, which 24 were IgM and 2 biclonals (IgM + IgG) (17.7 % of the MG series, 0.36% of the population). It was 21 males and 5 females, with a median age of 71 years (54-85 years). Distribution for light chain a show a clear predominance of the light chain k (20) opposite to light chain λ (6). 5 of these cases could not be studied because lack of consent or other social/sanitary circumstances, 1 case was a transitory Gammapathy and in 3 cases the Gammapathy was secondary to other diseases. Of 17 remaining patients, 13 (76 %) was catalogued of Monoclonal Gammapathy of Undetermined Significance (MUGS) and 3 (17.64%) of asymptomatic Waldenström’s Macroglobulinemia (aWM). No case of symptomatic Waldenström’s Macroglobulinemia. The patients with MGUS were 10 males and 3 women, with a median age of 76 years (56-85). The median of the component monoclonal by electrophoresis was 0.6 g/l (0.23-1.74). Median hemoglobin, serum creatinin and albumin levels were: 14.3 g/dl (11.6-15.4), 1.1 mg/dl (1-2) and 4.35 g/dl (4.4-4.9). From diagnoses, a patient has evolved to aWM. The patients with aWM were all males, with a median age of 71 years (56-80). The median of the component monoclonal was of 1.16 g/l (0.57-1.5). Median hemoglobin, serum creatinin and albumin levels were: 14.1 g/dl (10.1-15), 1.44 g/dl (1.0-1.5), 4.4 g/dl (4.0-4.6). Conclusions. In our population study, the proportion of IgM MG is similar to reported in other studies, although the incidence appears somewhat lower. These information will be updated in the meeting.

**0873**

**PERCUTANEOUS VERTEBROPLASTY IN MULTIPLE MYELOMA: A SINGLE CENTRE EXPERIENCE.**

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**Background.** Multiple myeloma (MM) is frequently associated with osteolytic lesions with bone pain and pathological fractures: vertebral compression fractures are present in 60% of patients at diagnosis. Percutaneous vertebroplasty (PVP) is a minimally invasive, radiologically guided procedure, in which bone cement is injected into destructed vertebrae. It has been shown to provide rapid pain relief and improve vertebral stabiility. However, in a fracture test, analgesic, bisphosphonates and radiotherapy fail (80% of cases). This study was conducted to test the efficacy and safety of PVP. Methods. A prospective analysis was performed on a total of 59 levels in 26 patients with vertebral pain refractory to conventional medical therapy for at least two months. Vertebral fractures were recognized when kyphotic deformity of ≥ 10° was documented on conventional X-rays. Percutaneous vertebroplasty was introduced: 24 levels in 12 patients, and 35 levels in 14 patients. In 6 patients, PVP was performed after radiotherapy. All the procedures were performed by two experienced neu-
The management of MM.

Ficacy and safety, early treatment with PVP should be considered in patients with refractory myeloma.

Evidence suggests that PVP is safe and effective and results in pain relief in 95% of patients (Oakervee, 2009).

Amethasone (PAD) is a highly effective induction agent with response rates of up to 95% (Oakervee, et al., BJH 2005; 129:755-62). We have previously shown the superior efficacy of PAD chemotherapy over VAD/VAD-like regimens by comparing response to PAD given following relapse to the response previously obtained with VAD/VAD-like regimens in the same patients. Aims. To demonstrate the efficacy of bortezomib, adriamycin and dexamethasone combination therapy in patients with refractory myeloma. Methods. This was a phase 2 study with 3 cohorts, of 23 patients. Cohort 3 was for patients refractory to VAD and who proceeded directly to PAD chemotherapy. Results. 23 patients (17 females, 6 males, median age 62 years) achieved <PR to a median IgM level for comparison) (p=0.0002 using the Wilcoxon signed rank test). Conclusions. Using a novel trial design, requiring only small numbers of patients we have shown that PAD is superior to VAD using two independent statistical methods.

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**0875**

**NO CORRELATION OF SYSTEMIC HYPOGAMMAGLOBULINEMIA AND IMMUNOSUPPRESSION OF THE SAME IMMUNOGLOBULIN CLASS WITH TIME TO FIRST TREATMENT AND OVERALL SURVIVAL IN WALDENSTRÖM’S MACROGLOBULINEMIA PATIENTS**

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**Background.** Systemic hypogammaglobulinemia (SH) is a very common finding in Waldenström’s Macroglobulinemia (WM). Low levels of IgG have been associated with disease progression. Specific polyclonal antibodies, that cause plasma cell proliferation in WM, were identified in the absence of deformity or body height loss. Involvement of the axial skeleton is common (35 male /35 female) at diagnosis were included in the study. Median age was 69 yrs (range 44-91). Analysis of IgG and IgA was performed using standard methods.

**Conclusion.** No correlation of systemic hypogammaglobulinemia (SH) and/or impaired IgM synthesis with time to first treatment (TTT) and overall survival (OVS) in a cohort of 70 WM patients (35 male /35 female) at diagnosis were included in the study. Median age was 69 yrs (range 44-91). Analysis of IgG and IgA was performed using standard methods.

**References.**

younger patients with multiple myeloma (MM). Although new drugs such as lenalidomide or bortezomib have been shown the promising results as induction treatment, many different type of induction treatment regimens still have been used. We evaluate the efficacy and safety of the short course of high dose dexamethasone and the response adopted PAD (Bortezomib, Adriamycin, Dexamethasone) or VAD (Vincristine, Adriamycin, Dexamethasone) induction chemotherapy in the newly diagnosed younger patients with MM.

Methods. 83 newly diagnosed patients with MM from 20 institutions received 2nd cycles of high dose dexamethasone followed by PAD or VAD chemotherapy according to the response to the initial high dose dexamethasone. The primary endpoint was complete response (CR) + near CR rate after ASCT. Among 83 patients enrolled this study from November 2009, 19 patients (23%) have been dropped out. This trial will be continued until total 210 patients will be enrolled. The trial is registered on National Cancer Institute website, number NCT01255514.

Results. Eighty three patients (41 male, 42 female) were enrolled (median age; 56). 17 (21%) light chain disease were included. 26 (31%) patients were D-S stage I and 51 (62%) were stage III. According to the ISS, 15 (18%) patients had stage I, 41 (49%) had stage II and 27 (33%) had stage III. 24 (29%) patients had abnormal cytogenetics. There were 32% del(17), 7% del17q, 18% t(4;14), 13% t(14;16) and 27% t(11;14) in FISH analysis. Among the 64 evaluable patients, CR + PR rate was 44% (28/64) after 2nd cycles of high dose dexamethasone therapy. 28 patients (44%) received subsequent to chemotherapy and 33 patients (52%) received PAD chemotherapy. Among the 42 patients finished VAD or PAD chemotherapy, CR + PR rate was 81% (34/42). 29 patients were finished ASCT until now. 11% (7/64) treatment related deaths were observed. The cause of death was disease progression (n=3) and infections (n=4). Conclusion. The short course of high dose dexamethasone and the response adopted PAD or VAD induction chemotherapy followed by ASCT showed acceptable response rates and acceptable toxicities. We will report update results of this trial.

Table 1.

8077 Efficacy and Safety of Bortezomib in Previously Treated Patients with Multiple Myeloma in Routine Clinical Practice: Slovak Experience
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Background. Bortezomib is the first proteasome inhibitor approved for the treatment of patients with relapsed/refractory multiple myeloma (R/R MM). Since April 2005 bortezomib is reimbursed for the treatment of patients with multiple myeloma (MM) in relapse settings in Slovakia. Aims. This large, retrospective, non-interventional, single-center analysis was conducted to evaluate bortezomib efficacy and safety in patients with R/R MM in routine clinical practice. Methods. 169 patients with MM who received bortezomib treatment after at least one prior therapy and have demonstrated disease progression at the biggest Slovakian myeloma center were evaluated in this study. The starting dose was 1.5 mg/m2 twice or once weekly, length of cycle 21 or 35 days, maximum 8 cycles. Bortezomib was administered as monotherapy, 2-drug (V, bortezomib + dexamethasone) or 3-drug (BDV, bortezomib + dexamethasone + doxorubicine, VMF, bortezomib + melphalan + doxorubicine, VCD, bortezomib + cyclophosphamide + dexamethasone) combination. Results. Demographic and baseline characteristics: median age: years (range) 67 (55 - 84); male sex: n (%) 68 (40); ISS I: n (%) 18 (11); ISS II: 112 (66); ISS III: 59 (23); one prior therapy: n (%) 104 (62); two prior therapies: 48 (28); three or more prior therapies: 17 (10). Treatment exposure: median treatment cycles: 6 (1 - 8); bortezomib monotherapy: n (%) 19 (11); 2- drug combination: 38 (23); 3 - drug combination: 112 (66). Overall response of R/R MM patients to bortezomib: ORR (CR + VGFR + PR): n (%) 143 (85); CR: 15 (9); VGFR: 71 (42); PR: 57 (34); MR: 10 (6); SD: 4 (2); PD: 10 (6). Safety: all grades of peripheral neuropathy (PN): n (%) 79 (47); Gr 3: PN 9 (5); no Gr 4; all grades of thrombocytopenia: n (%) 20 (12); Gr 3: 3 (2); no Gr 4; GIT toxicity Gr 1;2: n (%) 20 (12), other adverse events Gr 3,4: n (%) 4 (2), for combination of all adverse events: no AE: n (%) 67 (40), Gr 1: 86 (51), Gr 3: 16 (9) Outcomes median (95% CI): TTP: 23,1 (19,8-25,6) months; OS: 34,7 (27,4-inf) months. Depth of response was in significant association with long-term outcome (p<0.0001), graph 1. There were no statistically significant differences in TTP and OS between monotherapy, 2 - drug and 3 - drug combinations, Long-rank test: p=0,49 (TTP) resp. p=0,80 (OS). Conclusions. R/R MM patients treated with bortezomib had high overall response rates and survival outcomes comparable with previously published results from phase 3 clinical studies. The safety profile was predictable, manageable and similar to experience in clinical trials. Bortezomib is the headstone of efficacy concerning combination MM treatment in routine clinical practice.

0877 KYPHOPLASTY AS FIRST LINE TREATMENT FOR VERTEBRAL LESIONS DUE TO MULTIPLE MYELOMA: A SINGLE CENTER EXPERIENCE
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Background. The complications of spinal bone disease in multiple myeloma (MM) include local pain and vertebral compression fractures (VCF). The treatment of MM vertebral lesions traditionally involves radiotherapy (RT) combined or not with bed rest, bracing and analgesics with or without chemotherapy. Recently new minimally invasive interventional methods such as Vertebroplasty and Balloon Kyphoplasty (BKP) are applying to treat the bone lesions. BKP as compared to Vertebroplasty is a more efficient method for restoration of lost vertebral height with lower incidence of cement leakage. Aim. To determine the safety and the efficacy of the BKP in patients with vertebral fractures secondary to MM. To verify the role of BKP as treatment of choice for vertebral bone lesions instead of the traditional treatment with local radiotherapy. Methods. Patients with MM and vertebral fractures or osteolytic lesions were treated with BKP the last 2 1/2 years in our Department. Through minimal incisions, two special inflatable balloons were transpedicularly or extrapedicularly inserted and inflated in each treated VB. After appropriate inflation of the balloons a certain degree of vertebrae compression was reached. Therefore, the BKP technique followed the principle of introducing a balloon to push the bone fragments back into the vertebral body. The complication associated with BKP is the possible of cement leakage and distal embolization. Results. 15 patients (7 males, 8 females, median age: 68 years, range: 53-80) with bone metastases were treated with BKP. Of these, 5 patients had osteolytic lesions and 10 patients had bone destruction of the vertebrae. The average number of vertebrae treated with BKP was 1.6 (range: 1-3) per patient. Postoperative pain relief was achieved in 15 patients (100%) with immediate improvements in the functional status of the treated patients. Conclusion. BKP as compared to Vertebroplasty is a more efficient method for restoration of lost vertebral height with lower incidence of cement leakage.
Table 1. Comparison of BKP with RT.

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<tr>
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<th>BKP</th>
<th>RT</th>
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<tr>
<td>Pain control</td>
<td>90%</td>
<td>80-85%</td>
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<tr>
<td>Biomechanical stability</td>
<td>+++</td>
<td>+</td>
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<tr>
<td>Fracture reposition</td>
<td>Immediate</td>
<td>After 10-15 d</td>
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<tr>
<td>Onset of the analgesic effect</td>
<td>Immediate</td>
<td>After 10-15 d</td>
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<tr>
<td>Refracture (same vertebra)</td>
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<td>Neurological complications</td>
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of reposition of the VCF was obtained. Consequently a proper amount of PMMA (polymethylmethacrylat) cement was injected in every VB. Deformity correction was evaluated using standard calculation formula based on the fractured vertical height pre and post operatively. Pain degree was estimated before and after the procedure using the VAS score.

**Results.** 17 patients (4 male, 13 female, median age 67, range 45-79) with a total of 43 vertebral fractures or significant osteolytic lesions were treated. All 17 pts tolerated the procedure well. Postoperatively, all pts were admitted to the one-day clinic and all of them were discharged ambulatory the following morning. The deformity correction was 90% for the 23% of the VCF, 60-89% for the 61% and 30-59% for the rest of the reducable fractures. Six months later the correction was stable. For a median follow up time of 18 months (range 4-50) there were no early or late complications related to the technique cement extravasation, ARDS, relapse of the MM in the same VB or even refracture. All patients experienced immediate and stable pain relief (median VAS pro-post: 6-1) and improvement in the quality of life. No patient was in need of additional local radiation therapy in the reconstructed vertebrae. The comparison of BKP in our patients vs RT (historical data) are presented on table 1. Conclusions. Balloon Kyphoplasty is an effective and safe minimal invasive surgical procedure. The correction of the kyphosis by BKP results in significant biomechanical advantage. Rapid and significant relief of pain is achievable with BKP. BKP could substitute local radiotherapy as treatment of choice for vertebral lesions. Absence of local relapse may reflect a possible control of the disease by mechanical or functional intervention.

**Results.** Before Bortezomib administration, 4 out of 6 treatment naive MM patients had laboratory findings consistent with sensory neuropathy without any clinical symptoms. 11 out of 17 patients who had received prior chemotherapy presented peripheral neuropathy (4 had received thalidomide and 7 the VAD regimen). This was the fact that 3/4 patients with prior thalidomide exposure presented senso-rymotor PN. 10 out of 15 (66%) had laboratory findings of demyelination and 4/15 (27%) of axonal damage. 30 MM patients (79%) evaluated after Bortezomib initiation (median time to evaluation 5 months) presented treatment related neuropathy characterized mainly by numbness, paraesthesias, burnings and pain mainly in the lower extremities. In details 7/30 (23.3%) experienced sensory neuropathy, 1/30 (3.3%) motor neuropathy and 22/30 (73.3%) sensory motor neuropathy. 5/30 (16.6%) presented demyelinating neuropathy while 22/30 (73.3%) axonal and 3 (10%) demyelinating with secondary ax-onal degeneration. 3 out of 6 patients with WM(37.5%) presented pe-ripheral neuropathy characterized by sensory symptoms, gait disor-ders, reduced or absent tendon reflexes, loss of vibration and pin sensi-bility with predominance to the lower extremities. ENG evaluation re-revealed findings that were consistent with a sensory motor neuropathy of predominantly axonal type. Conclusions. ENG study is consistent with axonal degeneration neuropathy while in WM patients with primarily demyelinating sensory motor neuropathy. Bortezomib induced peripheral neuropathy is a sensory motor neuropathy of ax-onal type with predominance to the lower extremities, including mainly serious sensory symptoms. Preexisting peripheral neuropathy in MM patients before Bortezomib administration is common and should be taken into account. Baseline neurological evaluation in pa-tients with monoclonal paraproteinemia is important allowing early detection of peripheral neuropathy.
EORTC QLQ-C30 and QLQ-MY20 were collected at baseline, at the beginning of every 3rd cycle, and at study discontinuation. Five HRQoL measurement time points subsequent to baseline were assessed. Six out of 19 HRQoL domains were preselected based on clinical relevance: Global Quality-of-Life, Physical Functioning, Fatigue, and Pain from QLQ-C30 and Disease Symptoms and Side Effects of Treatment from QLQ-MY20. Clinically meaningful HRQoL improvements from baseline were assessed for individual treatment arms and classified as exceeding minimal important differences (MIDs). MIDs were determined through domain-specific standard error measurement (SEM) whereby positive values denote improvements in Global Quality-of-Life and Physical Functioning while negative values reflect improvements in Pain, Fatigue, Disease Symptoms and Side Effects of Treatment. Within group statistical significance tests (p≤0.05) were also conducted. HRQoL observations at discontinuation were carried forward to the next planned measurement time point; data with separate HRQoL measurements at discontinuation were also evaluated. Results. Data from all 459 randomized patients were evaluated. Clinically meaningful improvements from baseline were more frequently observed in patients receiving MPR-R than those receiving MP. HRQoL improvements were observed as early as Cycle 4. At Cycle 16, MID was achieved in 5 out of 6 domains (Global Quality-of-Life, Physical Functioning, Fatigue, Pain, and Disease Symptoms) in patients receiving MPR-R while only 2 out of 6 domains (Global Quality-of-Life and Pain) achieved MID in the MP arm. MID improvements were not reported in the MPR arm at Cycle 16 (Table). When compared to baseline, all 48 lenalidomide-related observations (6 induction-related measurements at cycles 4, 7 and 10 for MPR and MPR-R patients plus 2 additional measurements at cycles 13 and 16 for MPR-R patients during lenalidomide maintenance) at a minimum showed preservation of HRQoL across the six domains. Conclusions. Lenalidomide is generally well tolerated and when taken continuously, it prolongs progression-free survival in patients with NDMM. MPR-R elicited frequent and often sustained clinically meaningful improvements in patient reported HRQoL from baseline, thus showing a favourable balance between efficacy and HRQoL.

HEALTH RELATED QUALITY OF LIFE INSTRUMENTS FOR USE IN PEOPLE WITH MULTIPLE MYELOMA: A SYSTEMATIC LITERATURE REVIEW

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Background. Recent treatment advances in multiple myeloma have improved survival, although a cure remains elusive. Alongside improved survival emerges a need to better understand health related quality of life (HRQoL) across all disease stages and into survivorship. Yet few trials measure HRQoL in myeloma, and there is no consensus regarding the best instruments to use. We conducted two parallel systematic reviews to help address these issues. Aims. Review 1: To identify multidimensional HRQoL instruments for use in people with multiple myeloma, and describe their measurement properties. Review 2: To determine the domains of quality of life important to people with multiple myeloma. Methods. We conducted systematic literatures reviews searching MEDLINE, PsycINFO, EMBASE, CINAHL, BNI and AMED databases. Search terms were Myeloma OR Haematological Cancer OR Bone Marrow Transplant OR Palliative Care AND Quality of Life OR Patient Reported Outcomes OR Psychometrics OR Test Validity (and synonyms). We ran database searches on 28/9/10 with no limits by date or language, and supplemented these with manual searching of key journals and reference/citation searching of all included articles and relevant new articles. Review 1: Inclusion criteria were 1) any study developing, validating or using a multidimensional HRQoL instrument AND reported some appraisal of that instrument; 2) any study with myeloma specified in the published sample, including mixed haematological, cancer or palliative samples; and 3) studies with all participants over 18 years old at diagnosis. We extracted any psychometric or other appraisal of the HRQoL instruments, including predictive or prognostic properties. Review 2: Inclusion criteria were 1) any study identifying the domains of quality of life important to people with myeloma using methods such as (but not limited to) surveys, focus groups or interviews; 2) studies with samples entirely of myeloma patients; and 3) studies with all participants over 18 years old at diagnosis. Results. The database searches indentified 10,650 references. Review 1: 26 studies met the inclusion criteria, containing 10 different HRQOL instruments. Some instruments appeared only once, with the most frequent and extensively validated being the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30. We report the measurement properties of all included instruments. Review 2: 3 additional studies met the inclusion criteria for Review 2. This highlights the relative lack of published research exploring the issues important to quality of life in this group. Discussion. Myeloma patients can often fall within mixed samples used to validate other instruments (e.g. bone marrow transplant), or ‘area’ specific instruments (e.g. for use in cancer or palliative groups). Disease specific instruments may not be appropriate in settings such as palliative care, where validation of existing palliative instruments in myeloma patients may be more appropriate. Summary/Conclusion. There is a need for more research exploring the domains of HRQOL important to people with myeloma. The lack of such research has implications for the validity of existing HRQOL instruments in this group.
have demonstrated the cost-effectiveness of ZOL vs. CLO in this setting. However, in many countries, pamidronic acid (PAM) is the usual alternative to ZOL. Aims. To evaluate the cost-effectiveness of ZOL vs. CLO and ZOL vs. PAM in patients with newly-diagnosed MM. Methods. An economic model was used to project PFS, OS, the incidence of SREs and adverse events (ARF, ONJ, thromboembolism, and infection) as well as expected lifetime health-utility costs for patients with newly-diagnosed MM who are alternatively assumed to received ZOL 4 mg q 3-4 wk, CLO 1,600 mg/d PO, or PAM 90 mg IV q 4 wk x 9 cycles, in addition to CT. Cost-effectiveness was expressed in terms of incremental cost per quality-adjusted life-years (QALYs) gained with ZOL vs. CLO and ZOL vs. PAM. Estimates of OS, PFS, SREs, and incidence of AEs for ZOL and CLO were based on data from the MRC Myeloma IX trial. Hazard ratios (HRs) for SREs and AEs for PAM vs. ZOL were from the Phase III trial of ZOL vs. PAM. HRs for PFS and OS for PAM vs. CLO were from an adjusted indirect comparison of controlled trials of PAM and CLO vs. no bisphosphonate therapy. AEs with PAM were assumed the same as with ZOL. Costs (2009/10 Canadian dollars) and utility values were from published sources. Costs and QALYs were discounted at 5% annually. Results. Expected lifetime costs of bisphosphonate therapy (including administration and monitoring costs) were estimated to increase by $13,026 with ZOL vs. CLO ($15,530 vs. $2,504) and by $13,486 with ZOL vs. PAM. Expected costs of SREs were projected to be reduced by $720 with ZOL vs. CLO ($4,152 vs. $4,872) and by $324 with ZOL vs. PAM. Expected total lifetime costs were estimated to increase by $12,923 with ZOL vs. CLO ($32,088 vs. $19,165) and by $2,597 with ZOL vs. PAM. Life expectancy (undiscounted) was increased by 0.83 years with ZOL vs. CLO (6.43 vs. 5.60) and 0.28 with ZOL vs. PAM. Cost per QALY gained was $34,848 with ZOL vs. CLO and $44,272 with ZOL vs. PAM. Results were sensitive to methods used to estimate PFS and OS and the estimated HR for OS with PAM vs. CLO. Conclusions. In patients with newly-diagnosed MM, the cost-effectiveness of ZOL vs. CLO and ZOL vs. PAM is substantially below the generally-accepted threshold of $100,000 per QALY gained in Canada. Given this threshold, ZOL should be the preferred bisphosphonate treatment for patients with newly-diagnosed MM in Canada.

References

0883
COST-EFFECTIVENESS OF ZOLEDRONIC ACID VERSUS CLODRONIC ACID AND PAMIDRONIC ACID IN PATIENTS WITH MULTIPLE MYELOMA FROM A CANADIAN HEALTHCARE SYSTEM PERSPECTIVE
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Background. The Medical Research Council (MRC) Myeloma IX study demonstrated that the intravenous (IV) bisphosphonate, zolendronic acid (ZOL) 4 mg q 3-4 wk improves overall survival (OS) and progression-free survival (PFS) and reduces the incidence of skeletal related events (SREs) compared with the oral bisphosphonate, clodronic acid (CLO) 1,600 mg/d PO, in patients with newly-diagnosed multiple myeloma (MM) in addition to chemotherapy (CT). Previous analyses

0884
IMPROVED PROGRESSION-FREE AND OVERALL SURVIVAL WITH THALIDOMIDE MAINTENANCE THERAPY AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA: A META-ANALYSIS OF FIVE RANDOMIZED TRIALS
C Hahn-Ast, M von Lilienfeld-Toal, P van Heteren, S Mückter, P Brossart, A Glimacher
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Background. In two randomized studies, lenalidomide maintenance therapy after autologous stem cell transplantation (autoSCT) improved progression-free survival (PFS) but not yet overall survival (OS) in patients with multiple myeloma (MM). For thalidomide, more data are available and meta-analyses have been performed but the effect of maintenance therapy after autoSCT on OS remains unclear (Hicks et al. Cancer Treat Rev 2006, Hahn-Ast et al. EHA 2010). Recently, new studies or updates of previous data were published. Aims. We performed a new meta-analysis to evaluate the influence of thalidomide maintenance on OS in patients with MM after autoSCT. In addition, we also analysed PFS and toxicity. Methods. PubMed, the Cochrane Library and conference proceedings from ASH, ASCO, IMW, and EHA were searched using the headings “myeloma” and “thalidomide”, lastly in February 2011. Studies were included if they were randomized controlled trials (RCTs) of patients with MM receiving thalidomide maintenance therapy as monotherapy or combination therapy. Data were pooled using the random effects model. Measures of treatment effect were hazard ratios (HR) for survival data and relative risk (RR) for toxicity. Results. Expected lifetime costs were estimated to increase by $12,923 with ZOL vs. CLO ($32,088 vs. $19,165) and by $2,597 with ZOL vs. PAM. Life expectancy (undiscounted) was increased by 0.83 years with ZOL vs. CLO (6.43 vs. 5.60) and 0.28 with ZOL vs. PAM. Cost per QALY gained was $34,848 with ZOL vs. CLO and $44,272 with ZOL vs. PAM. Results were sensitive to methods used to estimate PFS and OS and the estimated HR for OS with PAM vs. CLO. Conclusions. In patients with newly-diagnosed MM, the cost-effectiveness of ZOL vs. CLO and ZOL vs. PAM is substantially below the generally-accepted threshold of $100,000 per QALY gained in Canada. Given this threshold, ZOL should be the preferred bisphosphonate treatment for patients with newly-diagnosed MM in Canada.

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All trials included only patients with newly diagnosed MM. In two trials thalidomide was also part of the induction regimen. PFS was significantly improved with maintenance thalidomide (HR 0.64, 95% CI 0.55-0.75, p<0.001). The effect was similar in trials with or without prior thalidomide-containing induction treatment. Interestingly, OS was also improved with maintenance thalidomide (HR 0.73, 95% CI 0.60-0.89, p=0.002). In the subgroup of trials with prior thalidomide induction, the effect also reached significance (HR 0.63, 95% CI 0.60-0.69, p=0.04). No significant heterogeneity among all RCTs existed between PFS or OS HRs (I²=51%, p=0.09 and I²=34%, p=0.20 respectively). Toxicity was not significantly different between thalidomide and comparators except for grade 3/4 neuropathy (reported in two trials), which was worse with thalidomide (RR 6.97, 95% CI 1.44-33.78, p=0.02). The rate of thromboembolic events (TE) grade 3/4 was reported in four trials. No significant difference was detected, although there was a trend to more thromboembolic events in the thalidomide arm (RR 2.01, 95% CI 0.96-4.23, p=0.07). Conclusion. In our meta-analysis we found an improved PFS and for the first time also an improved OS for thalidomide maintenance therapy after autoSCT in patients with MM. This effect was accompanied by a higher rate of grade 3/4 neuropathy whereas the rate of TE was not significantly increased. Supported by Celgene Pharma GmbH and Leukämie-Initiative Bonn e.V.

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Myeloma and other monoclonal gammopathies - Clinical 2

0885

IQ’KAPPA/ IQ’LAMBDAMEASUREMENTS IMPROVE DISEASE MONITORING AND IDENTIFY MINIMAL RESIDUAL DISEASE IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Currently monoclonal protein measurements are not used to prognosticate multiple myeloma (MM) patients; but they are important tools to assess responses to therapy. Traditional electrophoresis techniques (serum protein electrophoresis (SPE), capillary zone electrophoreses (CZE) and immunofication (IFE) are commonly used but the results generated can be inaccurate depending of the co-migration of the monoclonal protein with other serum proteins. Polyclonal antibodies recognising junctional epitopes between immunoglobulin light chains and their heavy chain partners (heavy/light chain [HLC]) have been developed. Aim. To assess the utility of HLC measurements to identify, prognosticate and monitor MM patients. Methods. HLC pairs (IgA κ and IgA λ, IgG κ and IgG λ) were retrospectively assessed in serial sera samples from 103 (38 IgGκ / 18 IgGλ and 31 IgAκ / 21 IgAλ) MM patients and results compared to historic markers of disease. Results. Patient characteristics were: median age 67 (range 52-94), 37 stage 1, 42 stage 2, 26 stage 3, median follow up was 27 months (0-158) with a median overall survival of 46 months (range 27-66). At presentation HLC ratios (HLCr) were abnormal in all 103 patients. In 36/103 patients (4 IgG and 32 IgA) MM patients with M-protein was not accurately quantifiable by CZE or SPE. Multivariate Cox regression analysis identified β2-M (p=0.01), LDH (p=0.004) and HLCr (p=0.049) as independent prognostic markers. A risk stratification model based on β2-M>3.5mg/L and abnormal HLCr (≤0.025 or >40) identified patients with 0, 1, or 2 risk factors and was associated with OS (median survival 131.2 v 53.6 v 29.2 months respectively; p=0.01). Throughout monitoring there was good correlation between percentage change in SPE and percentage change in involved HLC (Pearson’s correlation: IgA= 0.98 [p=0.0001]; IgG=0.9 [p=0.0001]). Following treatment, 13/103 patients (4 IgG and 9 IgA) achieved complete response (CR). HLCr remained abnormal in 4/13 patients who achieved CR. In 2/13 patients relapse of the disease was identified by HLCr before IFE (range 71-112 days), suggesting HLCr correctly identified residual disease that was undetectable by IFE. Interestingly, different responses were seen between FLC and HLC measurements in 3/103 patients: 1/3 patients relapsed with a FLC producing clone that was not identified by HLCr and in 2/3 patients FLC levels and ratios normalised at maximum response. In both cases the patients relapse was characterised by a clone producing only intact immunoglobulin. Conclusion. HLCr can detect hard to quantify IgA and IgG and low levels of both IgA and IgG paraproteins, indicate persistent disease in IF negative patients, develop.

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THE COMBINATION OF THE PROTEASOME INHIBITOR BORTEZOMIB WITH DOXORUBICIN AND DEXAMETHASONE (PAD REGIMEN) IS EFFECTIVE IN REVERSING RENAL IMPAIRMENT IN NEWLY-DIAGNOSED HIGH-RISK MULTIPLE MYELOMA

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Renal impairment (RI) due to light-chain induced nephropathy is a common presenting feature of multiple myeloma (MM), and is associated with increased morbidity and inferior survival. Reversion of RI is therefore essential for the management of MM. Among novel agents, bortezomib has shown promising efficacy in this context due to its an-

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treatment on renal function in a group of patients with high-risk MM, who received upfront therapy with a bortezomib-based regimen in a prospective phase II study. The study group consisted of 40 patients, aged 41-70 (median, 59) years, with newly diagnosed MM with high-risk features (ISS stage II/III by serum albumin and beta2-microglobulin, and/or detection of 13q deletion by karyotype or FISH). The treatment protocol included 4 cycles of the PAD regimen, i.e. the combination of bortezomib (1.3 mg/m² on days 1, 4, 8, and 11), doxorubicin (9 mg/m² on days 1-4), and dexamethasone (40 mg on days 1-4 and 8-11), administered every 21 days. Tumor response was assessed at the end of induction with PAD by the International Myeloma Working Group (IMWG) uniform response criteria (2006). Renal function was assessed at diagnosis and at the end of treatment with PAD by the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) study equation. Stage of RI was classified from stage 1 to 5 for eGFR values of ≥90, 60-89, 30-59, 15-29, and <15 ml/min/1.73 m², respectively. For the subgroup of patients with eGFR <50 at diagnosis, renal response to antimyeloma therapy was defined as complete (CRrenal), partial (PRrenal), or minor (MRrenal) according to the IMWG criteria (2010). All patients completed the 4 cycles of PAD, with the exception of one who died of pneumonia during the 2nd cycle. The overall myeloma response rate was 95%. Complete or very good partial remission (CR+VGPR) was achieved in 27/39 (69.2%), and partial remission (PR) in 10/39 (25.5%). At diagnosis, RI was mild (eGFR, 60-89) in 13/40, moderate (eGFR, 30-59) in 13/40, severe (eGFR, 15-29) in 4/40, and end-stage (eGFR <15) in 4/40 patients. ISS stage III, lambda light chain isotype, and high serum concentration of the involved free light chain were independent risk factors for higher stage of RI (p=0.018, 0.012, and 0.028, respectively). After the 4 cycles of PAD, a significant increase in eGFR was observed in comparison with baseline eGFR (median, 83 vs. 59 ml/min/1.73 m², respectively, p=10⁻⁷), and an improvement in the stage of RI was documented in 25/39 (64%) patients. Improvement in renal function was seen irrespective of the type of myeloma response (CR vs. VGPR vs. PR or worse, p=0.79). Among the 11 patients with pre-treatment eGFR <50, CRrenal was achieved in 4 (36%), PRrenal in 2 (18%), and MRrenal in 2 (18%). In conclusion, induction with bortezomib in combination to doxorubicin and dexamethasone resulted in overall improvement in renal function in patients with high-risk MM. Moreover, meaningful renal responses were achieved in more than half of patients with moderate to severe RI.
CARFILZOMIB PHARMACOKINETICS, SAFETY, AND ACTIVITY IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA AND RENAL DYSFUNCTION: FINAL RESULTS

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Background. Carfilzomib, a novel, highly selective epoxyketone proteasome inhibitor, has demonstrated single-agent activity in patients have not dramatically improved and disease-free survival (DFS) is only marginally superior. In available randomized trials, overall survival (OS) appears to be the same regardless of the first line pre-SCT induction treatment administered. Aims. Compare the outcome in terms of survival of two cohorts of patients with newly diagnosed MM and treated with different induction strategies. Patients and Methods. Data of two cohorts of patients with a newly diagnosed MM, treated in a single centre and submitted to ASCT in first response were collected. The first cohort (C1: 1999-2005) received dexamethasone, alkylating agents and anthracyclin-based induction before ASCT and bortezomib and/or IMIDs at relapse. The second cohort (C2: 2005-2009) received IMIDs and/or bortezomib first line and was submitted to ASCT in first response and were treated at relapse with the same or alternative drugs. All patients had at least one year follow-up after ASCT. Post-ASCT CR rates, time to progression (TTP), event-free survival (EFS), time to next treatment (TNT) and OS were determined for both cohorts to compare their outcomes. Results. Out of 141 potential ASCT candidates diagnosed during both periods, 88 received an ASCT after induction (N=49 in C1 and N=39 in C2). Their median age was 58 years (range 32-88). Both cohorts were comparable in terms of gender, age, type of myeloma and stage. Median time from diagnosis to ASCT was 34 weeks (range 14-80 weeks) without significant differences between cohorts. Post-ASCT CR rates were 35% for C1 and 61% for C2 (p=0.025). During the first year post-ASCT 6 patients died due to toxicity or infection (12%), 3 relapsed (6%) and 1 died of unrelated causes among 49 patients at risk in C1, during the same period, 1 patient died due to progression (2%) among 39 patients at risk in C2. During the second year post-ASCT 1 patient died due to toxicity or infection (2%) and 3 relapsed (8%) among 38 patients at risk in C1, while during the same period, no events were recorded among 36 patients at risk in C2. During the first year after SCT, the both probability of death of any cause (p=0.012) and the probability of infectious or toxic death (p=0.025) were significantly higher for C1. Differences in outcome, after a median follow-up of 6 years for C1 and 3 years for C2, are reflected in the following table. The projected post-ASCT for C2 at 4 years was 83% (95% CI 65-98%). Conclusion. In our experience, patients with MM who received chemotherapy as first line induction presented an increased risk of death due to infections or toxicity during the first year post-ASCT compared with those receiving bortezomib or thalidomide first line. Post-ASCT CR rate and survival of patients were superior for the cohort of patients treated with bortezomib or thalidomide as pre-ASCT induction.

Supported in part with grants P-EF-09 from FJJC and RDO6/0020/1056 from RTICC.

Table 1.

<table>
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<tr>
<th>Carfilzomib Use</th>
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Figure 1

Prognosis of multiple myeloma (MM) patients after autologous stem cell transplantation (ASCT) in the last decade. Comparison of two cohorts with different induction treatment approaches

A. Oriol,1 JM Ribera,1 M Balle,1 I Llombart,1 C Moliar1 Y Gonzalez,2 S Vives,3 C Ferr3 B Xico,4 J Sancho5

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Background. The incorporation of bortezomib and IMIDs to MM treatment has improved substantially improved pre-SCT overall and complete remission (CR) rates in candidates to SCT. However, post-SCT results have not dramatically improved and disease-free survival (DFS) is only marginally superior. In available randomized trials, overall survival (OS) appears to be the same regardless of the first line pre-SCT induction treatment administered. Aims. Compare the outcome in terms of survival of two cohorts of patients with newly diagnosed MM and treated with different induction strategies. Patients and Methods. Data of two cohorts of patients with a newly diagnosed MM, treated in a single centre and submitted to ASCT in first response were collected. The first cohort (C1: 1999-2005) received dexamethasone, alkylating agents and anthracyclin-based induction before ASCT and bortezomib and/or IMIDs at relapse. The second cohort (C2: 2005-2009) received IMIDs and/or bortezomib first line and was submitted to ASCT in first response and were treated at relapse with the same or alternative drugs. All patients had at least one year follow-up after ASCT. Post-ASCT CR rates, time to progression (TTP), event-free survival (EFS), time to next treatment (TNT) and OS were determined for both cohorts to compare their outcomes. Results. Out of 141 potential ASCT candidates diagnosed during both periods, 88 received an ASCT after induction (N=49 in C1 and N=39 in C2). Their median age was 58 years (range 32-88). Both cohorts were comparable in terms of gender, age, type of myeloma and stage. Median time from diagnosis to ASCT was 34 weeks (range 14-80 weeks) without significant differences between cohorts. Post-ASCT CR rates were 35% for C1 and 61% for C2 (p=0.025). During the first year post-ASCT 6 patients died due to toxicity or infection (12%), 3 relapsed (6%) and 1 died of unrelated causes among 49 patients at risk in C1, during the same period, 1 patient died due to progression (2%) among 39 patients at risk in C2. During the second year post-ASCT 1 patient died due to toxicity or infection (2%) and 3 relapsed (8%) among 38 patients at risk in C1, while during the same period, no events were recorded among 36 patients at risk in C2. During the first year after SCT, both the probability of death of any cause (p=0.012) and the probability of infectious or toxic death (p=0.025) were significantly higher for C1. Differences in outcome, after a median follow-up of 6 years for C1 and 3 years for C2, are reflected in the following table. The projected post-ASCT for C2 at 4 years was 83% (95% CI 65-98%). Conclusion. In our experience, patients with MM who received chemotherapy as first line induction presented an increased risk of death due to infections or toxicity during the first year post-ASCT compared with those receiving bortezomib or thalidomide first line. Post-ASCT CR rate and survival of patients were superior for the cohort of patients treated with bortezomib or thalidomide as pre-ASCT induction.

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EVALUATION OF EFFICACY AND SAFETY OF BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE (PAD) REGIMEN IN PATIENTS WITH RELAPSED AND REFRAC TORY MULTIPLE MYELOMA


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Background. Multiple myeloma is a malignant plasma cell disorder. It is the second most frequent haematological malignancy and characterized by malignant plasma infiltration of the bone marrow and is associated with an increased level of monoclonal protein in the blood and urine. The treatment of multiple myeloma (MM) has undergone significant developments in recent years. The development of new agents with potent anti-tumor activity has considerably improved the survival of MM patients. Bortezomib has been investigated as part of a number of different induction regimens. A randomized phase III study by the French Myeloma Study Group (Institut Français du Myéline [IFM]) examined the combination of bortezomib plus dexamethasone and found this to be significantly superior to the comparator arm, which consisted of VAD, with respect to response rates postinduction and post-transplant, as well as the 2-year PFS rate. A number of bortezomib induction regimens are now available. The results of the IFM trial indicate that the combination of bortezomib and dexamethasone is an appropriate regimen that is superior to the traditional VAD regimen. Aim. Evaluation of the effect and safety of combination of bortezomib, doxorubicin and dexamethasone (PAD) in the treatment of relapsed/refractory myeloma patients in the retrospective analysis. Patients and Methods. 101 patients were treated for median of four 28-day PAD cycles (1-8). Bortezomib was given at 1.5 mg/m² (days 1, 4, 8, 11), doxorubicin at 9 mg/m² (days 1-4) and dexamethasone 20 mg po (days 1-4, 8-11). Results. 101 patients were evaluable for efficacy and safety. 63.2% had refractory disease and 36.8% were relapsed. The median age was 60 years (41-78), 45.5% were male, 54.5% female. Median time from diagnosis was 12 months (1-139) and median number of prior therapy lines was 1 (1-4). 90.9% had undergone thalidomide-base regimen, 9.1% conventional chemotherapy and 23.9% were autografted. Overall response rate of 77.4% was observed, 32.3% of patients achieved a complete response (CR), 19.8% a very good partial response (VGPR), 24.8% a partial response (PR), Stable disease (SD) was observed in 15.8%. After PAD 42.9% of patients were autografted. The median progression free survival (PFS) was 19.3 months. The probability of 2-years overall survival (OS) was 68.7% and the median of OS was not reached. The most common grade 3-4 toxic effects were neutropenia 14%, thrombocytopenia 15%, anemia 4.3%, infections 5.4%, peripheral neuropathy 4.3% and gastrointestinal disturbances 2.1%. One toxic death (1.1%) due to sepsis was noted. Conclusion. The combination of bortezomib, doxorubicin and dexamethasone (PAD) is well tolerated and induced clinically significant responses and prolonged remission duration in patients with relapsed and refractory MM.

OVERALL SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION: RELATION WITH KINETICS OF NEUTROPHIL AND PLATELET RECOVERY

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Background. Autologous stem cell transplantation (ASCT) is the gold standard as first-line treatment in young patients with multiple myeloma (MM). Prognostic factors have been usually related to patient characteristic and disease stage. Few investigations on the impact of...
platelet and neutrophil recovery after ASCT have been addressed. The aim of this study was to investigate the prognostic influence of the kinetics of peripheral blood recovery on progression-free survival (PFS) and overall survival (OS) after ASCT. Patients and Methods. One hundred and ninety-one patients (109M/82F; median age 55 years) underwent melphalan-based ASCT in our institution from 1994 to 2010. The median follow-up after ASCT was 4 years (range 4 months to 17 years). Peripheral blood recovery was assessed as the day when the neutrophils reached 500 (N500) and 1,000x10^9/L (N1000) and platelet count 20,000 (P20) and 50,000x10^9/L (P50) after CD34+ infusion. Patients were classified in two groups according their recovery above or below the median. Results. N1000 (Figure, P20 and P50 predicted for a longer OS (p<0.05). No significant association with the number of infused CD34+ cells was observed. PFS was also correlated with N1000 (Figure) (p=0.001) and there was a trend for N500 (p=0.053), with no impact of P20 and P50. In Cox multivariate regression analysis, including age, international staging system and immunoglobulin isotype, N1000 remained at significant level for PFS (p=0.02), and there was a trend for OS (p=0.09). Finally, a stratification model showed 3 prognostic stages according to the achievement of complete remission and early N1000 after ASCT (p<0.001). Conclusion. Early neutrophil recovery was associated with significantly longer PFS and OS, while platelet recovery was only associated with OS.

**0894**

**VORINOSTAT PLUS BORTEZOMIB COMBINATION THERAPY IN PATIENTS WITH RELAPSED OR RELAPSED AND REFRACTORY MULTIPLE MYELOMA: VANTAGE STUDY PROGRAM UPDATE**

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Background. Vorinostat is an oral multi-histone deacetylase inhibitor approved for the treatment of cutaneous T-cell lymphoma who have progressive, persistent, or recurrent disease following 2 prior systemic therapies. Vorinostat regulates genes and proteins involved in tumor growth and survival. The synergistic effects of vorinostat and bortezomib have been shown in preclinical studies and confirmed in phase I trials in patients with relapsed/refractory multiple myeloma (MM), producing objective response rates (ORR) of up to 42% and overall clinical benefit of up to 90%. Aims. To provide an enrollment update and demographic data for patients with relapsed/refractory MM participating in the Vantage clinical trial program. Methods. Vantage 088 is a global, phase III, randomized, double-blind study investigating bortezomib plus vorinostat or placebo in patients with relapsed MM and progressive disease after 1-3 prior regimens. The primary objective is determination of progression-free survival; secondary objectives include assessment of safety, overall survival, time to progression, and ORR. Vantage 095 is a phase IIB open-label study of vorinostat plus bortezomib in bortezomib-refractory patients with relapsed/refractory MM who had received ≥2 prior anti-lymphoma regimens and were relapsed, refractory to, intolerant of, or ineligible for other MM therapies, including immunomodulatory drugs. The primary objective is to determine the ORR. In both studies, patients receive 21-day cycles of bortezomib (1.3 mg/m² IV; days 1, 4, 8, and 11) plus oral vorinostat 400 mg/day (or matching placebo in Vantage 088) on days 1-14. Efficacy is assessed by European Bone and Marrow Transplantation Group criteria. Adverse events, including clinical and laboratory events are assessed and recorded using the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). All patients in both studies gave written informed consent prior to enrollment. Results. Both trials have been fully enrolled (Vantage 088 in January 2011; Vantage 095 in October 2010). As of February 10, 2011, 185 of 637 patients in Vantage 088 and 15 of 143 patients in Vantage 095 were still on active study therapy. Vantage
and chosen MM therapy. Recommended Phase II dose of 10 mg/kg is supported with current data predicting maximal saturation and suppression of DKK1. BMD changes > 6% from baseline in 33% of patients and changes in bone markers in this patient population suggest a potential anabolic effect.

0896 AN OPEN-LABEL PHASE 1/2 TRIAL OF BENDAMUSTINE PLUS BORTEZOMIB IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Background. Although significant improvements in the understanding and treatment of multiple myeloma (MM) have been realized over the last decade, MM remains an incurable disease, and novel, effective treatment combinations are needed for patients with relapsed/refractory disease. Bendamustine, approved for the treatment of MM, is an alkylating agent with a multifaceted mechanism of action. Bortezomib is a proteasome inhibitor approved for the treatment of MM that has demonstrated efficacy in combination with alkylators (e.g., melphalan and cyclophosphamide). Aims. To assess the efficacy and safety of bendamustine plus bortezomib in patients with relapsed or refractory MM. Methods. This open-label, phase 1/2 study enrolled patients ≥18 years, with measurable, relapsed or refractory MM; patients were required to provide informed consent. Escalating doses of bendamustine IV at 50, 70, or 90 mg/m² (days 1 and 4) plus bortezomib at a fixed dose of 1.0 mg/m² (days 1, 4, 8, and 11) were administered for up to eight 28-day cycles. Dose-limiting toxicity (DLT) was assessed after cycle 1. A standard 3+3 approach was used to determine the maximum tolerated dose (MTD). The MTD cohort was expanded until a total enrollment of 40 patients was reached. Study endpoints included response, duration of response (DOR), time to progression (TTP), and safety. Results. Thirty-eight patients (median age, 67; range, 43-89) received study drug and were included in the analysis. Patients received a median of 3.5 (range, 1-21) prior therapies, including bortezomib in 71% and alkylators in 68%. A median of 3 treatment cycles (range, 1-9) were administered; study treatment remains ongoing in 14 patients (median cycles to date: 4 [range, 1-7]). No DLT was observed in phase 1 and the MTD was not reached; thus, the maximum dose (90 mg/m²) of bendamustine plus bortezomib 1.0 mg/m² was studied in phase 2. Grade 3/4 adverse events that occurred in ≥10% of patients were neutropenia (15 patients [34%]), thrombocytopenia (7 [21%]), and anemia (4 [11%]). Grade 3/4 infection was reported in 3 patients (8%) and grade 3 renal failure in 2 patients (5%). No grade 3/4 peripheral neuropathy (PN) was observed; grade 1/2 PN was reported in 10 patients (26%), but 8 of these had grade 1/2 PN at baseline. Among 36 evaluable patients, the objective response rate (ORR) was 47%, including 11 very good partial response, 6 partial responses, and 10 minimal responses. An additional 17 patients had stable disease for a clinical benefit rate of 94%. In patients who received bendamustine at the 90 mg/m² dose (n = 27), the ORR was 52%. In patients previously treated with bortezomib (n = 27) or alkylators (n = 26), the ORR was 37% and 40%, respectively. Median DOR and TTP have not been reached. Conclusions. The combination of bendamustine and bortezomib demonstrates promising efficacy in heavily pretreated patients with MM.
A SYSTEMATIC REVIEW ON THE USE OF BORTEZOMIB IN MULTIPLE MYELOMA PATIENTS WITH RENAL IMPAIRMENT: WHAT IS THE PUBLISHED EVIDENCE?


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Background and Aims. A systematic and comprehensive search of the literature was performed using MEDLINE databases from 1978 to 1st December 2010 and hand search of references. We used the following Medical Subject Headings (MESH) to identify potential studies: 'myeloma renal failure' (365 results) and 'bortezomib' (365 results). An additional search performed by combining the MESH terms 'myeloma renal failure' and 'bortezomib' yielded 80 citations. Five additional case-control studies judged relevant for the purpose of study were also included. Methods. In total, 6 case reports, 9 cases series, and 9 case control studies were identified that reported on myeloma, renal failure and bortezomib. Only case series and case control studies were considered. We formulated some key questions dealing with reversal of renal impairment (RI), doses and association of bortezomib therapy as well as toxicity. Results. Overall 877 patients were considered suitable for this analysis. Median age was 65 years (range 40-88) and M/F ratio 1:0.2 whereas male patients had related/refractory MM (597 out of 877 or 68%) and 79% were in Durie& Salmon stage III. Heterogeneous were methods used for assessing the extent of RI. Serum creatinine concentration has been used in 6 studies and CrCl in 12. Another relevant issue concerns the heterogeneity of threshold selected to define RI. A CrCl value of 30 ml/min was used in 3 studies, between 50 and 60 ml/min in 5 additional reports, while CrCl cutoff was increased to 80 ml/min in a single study. Even definition of reversal of RI shows differences across studies. In 7 reports reversal of RI meets criteria of a reduction of creatinine levels. A more accurate method to define the degree of reversal of RI based on Ludwig criteria has been used in 4 recently published studies. Overall response rate after treatment with bortezomib was relatively high and did not reflect the pre-treatment renal status. Time to reversal of RI assessed in 10 reports appears to be relatively short (median 1.4 months; range from 0.5 and 3.7 months) and led to the dialysis independence 8 out of 52 (25%) previously dialyzed patients. Almost all patients were treated according standard schedule at dose of 1.3 mg/m2 2 days on 1, 4, 8, and 11. Repeated every 21 days. Adverse events and side effects were described in all 18 studies considered. Remarkably toxicity was generally found comparable in 5 studies including MM patients with and without renal failure. Conclusion. Despite the paucity of data, this article represents the first systematic review of the entire body of available clinical evidence dealing with the use of bortezomib in MM patients with RI.

FREE LIGHT CHAIN CLONAL ESCAPE IS USEFUL FOR EARLY DETECTION OF RELAPSE IN INTACT IMMUNOGLOBULIN MULTIPLE MYELOMA

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Background. Serum free light chains (sFLC) and ratio (sFLCR), are used for the evaluation of sCR in intact immunoglobulin (Ig) MM. Their use is not established for follow up and treatment initiation in intact Ig Multiple Myeloma (MM). Aim. To investigate the importance of sFLC and sFLCR elevation for the detection of clinical relapse, in the absence of any other clinical and laboratory finding. Patients and Methods. 51 intact immunoglobulin MM patients were studied from diagnosis last follow up. Their sera samples (n=312) were analyzed for sFLC quantification using Freelite® immunoassay. Results. Median lines of therapy were 2 (range 1-11). Median follow up was 28 months (4-135). Retreatment was initiated in all patients according to standard criteria. In 8/51 patients during remission (2 in plateate, 1 in MR, 1 in PR, 5 in nCR and 1 in sCR) only sFLC and sFLCR increased gradually (light chain clonal escape) and shortly after they relapsed, 2 acute renal failure, 1 acute renal failure and rise of paraprotein, 2 plasmacytomas, 1 liver plasmacytoma and rise of paraprotein, 1 rise of paraprotein and 1 plasmacytosis. Median time from onset of light chain clonal...
escape to clinical relapse was 6 months (2-11 months). Conclusion. Light chain clonal escape was observed during disease course in 15% of patients with intact Ig MM; shortly after they relapsed. Measurement of sFLC during follow up is useful for early detection of relapse in a subset of patients.

0900 PANORAMA 2: A PHASE II STUDY OF PANOBINOSTAT IN COMBINATION WITH BORTEZOMIB AND DEXAMETHASONE IN PATIENTS WITH RELAPSED AND BORTEZOMIB-REFRACTORY MULTIPLE MYELOMA

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Background. Many patients with multiple myeloma (MM) do not respond to currently available bortezomib-based therapeutic strategies and novel combinations are urgently needed. Panobinostat is an oral pan-deacetylase inhibitor (pan-DACi) with demonstrated synergistic anti-myeloma activity in combination with bortezomib through dual inhibition of the aggresome and proteasome pathways. In the doseescalation phase of a phase Ib study (B2207) of panobinostat + bortezomib, responses of ≥ minimal response (MR) of 76% (86/47) were observed. Of note, a ≥ MR rate of 66% (10/15) was observed among bortezomib-refractory patients. Based on these data, a multicenter, U.S.-based, single-arm phase II trial was initiated to further evaluate panobinostat + bortezomib (intravenous 1.3 mg/m2 day 1, 8, 22, 29) + dexamethasone (20 mg on day of and day after each bortezomib day). Patients demonstrating clinical benefit can be escalated to 2 weeks out of 3; matching the dosing schedule for bortezomib. PANORAMA 2 study, the dosing schedule of panobinostat is modified originally dosed in all 3 weeks of the treatment cycle. In the B2207 study, panobinostat was used in relapsed and bortezomib-refractory MM. In B2207, panobinostat was used in relapsed and bortezomib-refractory patients. Based on these data, a multicenter, U.S.-based, single-arm phase II trial was initiated to further evaluate panobinostat in patients with relapsed and bortezomib-refractory MM to a bortezomib-based therapeutic regimen. Methods. Adult patients with relapsed and bortezomib-refractory MM (≥ 2 prior lines of therapy including an immunomodulatory drug and who had progressed on/at least 80 days of last bortezomib-based therapy) are eligible. Treatment is comprised of two phases. Phase 1 consists of 8 three-week cycles of panobinostat (20 mg days 1, 3, 5, 8, 10, 12, 14, thrice weekly (TWW) 2 wks on 1 wk off) + bortezomib (intravenous 1.3 mg/m2 days 1, 4, 8, 11) + dexamethasone (20 mg on day of and day after each bortezomib day). Patients demonstrating clinical benefit can proceed to treatment phase 2 which consists of 4 six-week cycles of panobinostat (20 mg TWW 2 weeks on 1 week off, and repeat) + bortezomib (intravenous 1.3 mg/m2 day 1, 8, 22, 29) + dexamethasone (20 mg on day of and day after each bortezomib day). A Simon two-stage design will be used to test for the primary endpoint of overall response rate (≥ partial response) with ≥ 4 responses required to proceed to Stage 2. Secondary endpoints include MR rate, time to and duration of response, progression-free survival, time to progression, overall survival, and safety, including assessment of peripheral neuropathy. Results. As of Jan 24, 2011, 24 patients were enrolled and responses have been seen in bortezomib-refractory patients, with manageable toxicity reported to date. Further assessment for response is ongoing and additional evaluation for toxicity is in process. Summary/Conclusions. Enrollment to Stage 1 has been completed and interim analysis is underway, with Stage 2 expected to start soon. Updated demographic information along with preliminary safety and efficacy data will be presented at the meeting.

0901 BORTEZOMIB PLUS DEXAMETHASONE (VD) FOLLOWED BY AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) PRODUCES MOLECULAR REMISSIONS (MOLR) IN 23% OF PATIENTS WITH NEWLY DIAGNOSED MULTIPLE MYELOMA (MM)

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Background. The depth of complete remission (CR) may be critical for the long-term outcome of patients with MM. Thus, MoR is proposed as the new goal of treatment. Aim. The aim of this Finnish study was to explore the response rates after VD induction followed by ASCT, including minimal residual disease (MRD) assessment in patients with at least near-CR response. Methods. Until Dec 2010, 35 symptomatic MM patients with a median age of 61 (53-65) years with informed consent have been included. Study protocol consists of induction with four cycles of VD followed by ASCT with melphalan 200mg/m2 Patients were evaluated first time for response after two cycles of induction treatment. Those who had not attained at least partial remission (PR) were taken out of protocol treatment. In case of progressive disease patients were also out of protocol. MRD was assessed by allele specific quantitative polymerase chain reaction (PCR) using pretreatment sample with proportion of myeloma cells determined by flow cytometry as the reference. Results. After VD induction nine patients (26%) were in nCR/CR; two of them were PCR negative (sensitivity 0.003%-0.01%) and four PCR positive (range 0.002%-0.2%). Three patients had inadequate samples. Sixteen patients (46%) were in very good partial remission (VGPR) or in PR, and seven (20%) patients had response less than PR. Three patients are not yet evaluable. Two more patients were PCR-negative (sensitivity <0.001%) after mobilisation. Eighteen patients have undergone ASCT. Three months after ASCT, 12 patients (34%) are in nCR/CR, four in VGPR/PR, 10 patients not yet evaluable and nine patients out of study. Five patients are out of study, because their response was less than PR after two induction cycles, two patients had progressive disease, and two had severe adverse event (thromboembolic events with treatment delay). Of the 12 patients in nCR/CR, six are PCR-negative (sensitivity 0.001%-0.007%) and six PCR-positive (range 0.005%-0.09%). Two more patients have achieved PCR-negativity six months after ASCT. In all patients, except one, PCR-negativity was confirmed with two consecutive samples. Conclusion. Even with the relatively short median follow-up time PCR-negativity has already been achieved in 23% of the patients. Longer follow-up is needed to study if molecular remission is associated with beneficial long-term outcome.

0902 CHROMOSOMAL ABNORMALITIES AMP(1Q21) AND DEL(13Q14) PREDICT SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA TREATED WITH CTD REGIMEN

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Background. Chromosomal abnormalities are frequently found in multiple myeloma(MM) and play a major role in patient outcome and management of the disease. The most important chromosomal aberrations associated with worse outcome are del(17p13), (4,14), (14,16) and t(14,20). Others that may be associated with worse prognosis include del(13q14), del(8p21), amp(q12) and hyperdiploidy. In the era of novel agents such as thalidomide, lenalidomide, and bortezomib, risk stratification by chromosomal abnormalities may enable rational therapeutic approach in patients with MM. Aim. The study aimed to investigate the influence of amp(q12) and del(13q14) on survival of MM patients treated with CTD (thalidomide, cyclophosphamide and dexamethasone) regimen. Methods. We analyzed the prognostic value of del(13q14) and amp(q12) by fluorescence in situ hybridization (FISH) and hyper- (H-MM) or hypodiploidy (NH-MM) by conventional cyto-
HOSPITAL COSTS DURING TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA IN THE ERA OF TARGETED THERAPIES: REAL-WORLD ESTIMATES FROM THE NETHERLANDS

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Background. Targeted therapies for multiple myeloma (MM), such as thalidomide and bortezomib, promise gains in health but come at increased acquisition costs. Newer targeted therapies for treatment of MM are expected in the near future, and assessment of cost-effectiveness may be required for decision-making regarding their use. In the Netherlands, treatment with targeted therapies has until recently been confined to relapsed/refractory disease (RRMM). We conducted a cost study to estimate the total costs and drivers of increased costs during treatment for RRMM in the era of targeted therapies in the Netherlands. Aims. To describe the costs of treatment for RRMM and determine whether there are differences between the treatment costs of thalidomide and bortezomib. Methods. Patients in this cohort represented a subset of 189 patients treated in the HOVON50 study, which was a phase III trial comparing VAD (vincristine, adriamycin and dexamethasone) with TAD (thalidomide adriamycin dexamethasone) in first-line treatment of MM. In this subset, 65% (n=90) progressed from the VAD treatment arm compared to 35% (n=49) from the TAD treatment arm, respectively. Detailed clinical data were retrospectively collected for each patient up until last known follow-up between January 2001 and May 2009. Total costs for individual patients were determined by the identification of hospital resource use and unit costs of all cost components. Monthly resource use and costs attributable to each cost component were described across all treatment lines and separately by treatment line. Results. Results were also calculated for thalidomide- and bortezomib-based treatment regimens. Results. The combination of treatment regimens and sequence of administration throughout treatment of RRMM varied greatly. Total mean follow-up of the patient group equaled 24 months (range: 6-70.1) with 49% of patients still in follow-up at the time of data collection. In total, 87 patients were treated with thalidomide and 72 with bortezomib, with some patients receiving either therapy more than once as well as concurrently in combination during treatment of RRMM. Mean total monthly costs for treatment of MM patients in the Netherlands were approximately €1,109 and the minimum-maximum range was large (€442-€1,740). Mean total monthly costs did not differ significantly between patients still in follow-up and those with complete follow-up. The structure of the total costs incurred during treatment differed between bortezomib and thalidomide treatment lines. Total mean monthly acquisition costs for bortezomib were higher compared to thalidomide (€2,574 vs €955.8 mg vial of bortezomib and €0.24/mg of thalidomide). Moreover, monthly costs associated with adverse events and drug administration were higher during bortezomib-based treatment regimens compared to thalidomide-based treatment regimens. Summary/Conclusions. The costs of treatment during RRMM are substantial and vary depending on the order in which therapies are given. Cost-effectiveness analyses of targeted therapies for MM should take into account the impact of increased therapy-related costs on the total treatment costs. Furthermore, the costs of potentially avoidable adverse events associated with specific treatment regimens should be considered when determining the most cost-effective treatment sequence for RRMM.
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Background. Bortezomib-dexamethasone-doxorubicin (PAD) induction, followed by reduce-intensity autologous transplantation proved to be safe and effective in elderly newly diagnosed multiple myeloma (MM) patients. Lenalidomide is less neurotoxic than thalidomide and represents an optimal agent to include in maintenance strategies. Aims. This sequential approach including bortezomib as induction and lenalidomide as consolidation-maintenance in elderly MM patients undergoing reduced-intensity ASCT.

Methods. A hundred and two newly diagnosed patients, aged 65-75 years or younger not eligible for high-dose chemotherapy, were enrolled. Patients received PAD induction, tandem melphalan 100 mg/m2 and stem-cell support followed by consolidation with four 28-day cycles of lenalidomide-prednisone (LP-L), and subsequent lenalidomide maintenance (L) until relapse or until tolerated. Results. LP-L consolidation-maintenance therapy improved post transplantation responses: VGR rate raises from 82% to 92%, complete response (CR) rate from 38% to 71%; 1 patient improved from stable disease (SD) to partial response (PR), 1 from SD to very good partial response (VGPR), 1 from PR to complete response (CR), 1 from PR to very good partial response (VGPR), 1 from PR to complete response (CR), and 1 from SD to partial response (PR).

Conclusions. This sequential approach improved depth and rate of response, was well tolerated, and can be considered a safe and effective treatment strategy for elderly MM patients eligible for reduced-intensity ASCT.
dent between responses and JAK2 or MPL mutational status or cytogenetics. Pomalidomide with or without prednisone may be effective in the treatment of cytopenia in patients with MPN-associated myelofibrosis. Most responses were seen during combined therapy with pomalidomide and prednisone beyond month 3 of treatment.

**0907**

LONG-TERM SAFETY AND EFFICACY ANALYSIS OF THE TWO PHASE 1 STUDIES OF SB1518, A NOVEL ORAL JAK2 INHIBITOR, IN PATIENTS WITH ADVANCED MYELOID MALIGNANCIES

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**Background.** SB1518 is a potent inhibitor of both wild-type JAK2 and JAK2V617F, implicated in the pathogenesis of myeloproliferative neoplasms. In mid-2008, we initiated two Phase 1 studies of SB1518: one in patients with advanced myeloid malignancies, and the other ongoing in patients with myelofibrosis (MF). We previously reported the study data that supported 400 mg/d as the recommended Phase 2 dose (RD). These trials are ongoing, as some patients continue to receive SB1518.

**Aims.** To report the overall safety and efficacy results for the combined study populations as of January 2011. **Methods.** Qualifying patients with MF had palpable splenomegaly ≥ 5 cm. Patients were sequentially assigned to doses from 100-600 mg/d and were dosed continuously. Intra-patient dose escalation was allowed up to the RD once the MTD was established. **Results.** Sixty-three patients were consented and enrolled; 39 (62%) were men, and median age was 65.5 years. Fifty-six had MF, and 7 had AML. Fifty (79%) were JAK2V617F mutation-positive (46 MF, 4 AML). At baseline, hemoglobin ranged from 5.6 to 16.3 g/dL (median, 9.4). Platelet count ranged from 5 to 954 x 10^9/µL (median, 122 x 10^9/µL) with 16 patients having baseline counts <50 x 10^9/µL, 12, 50-100 x 10^9/µL, and 35; <150 x 10^9/µL. Median time on study is 13.3 months (1-29). As of January 2011, 21 MF patients remain on study. The most common treatment-related AEs were gastrointestinal, which were generally low grade and manageable. GI AEs > Gr 2 included 5 patients with Gr 3 diarrhea (7.9%) and 1 patient each (1.6%) with Grade 3 nausea, Grade 3 abdominal pain, and Grade 4 vomiting. Grade 4 hematologic AEs considered possibly related to treatment were rare and comprised anemia (n=2, 100 & 200 mg), and thrombocytopenia (n=1, 500 mg). These events occurred after 3.7-18.5 months on study. Two patients were discontinued for these AE’s and one continues on study at 200 mg dose. Fifteen patients had dose reductions, most within the first 6 months; of these, 11 (73%) started treatment at ≥500 mg/d. No patients discontinued study medication because of a dose-limiting toxicity. No long-term toxicities were identified. Forty-one MF patients had palpable baseline splenomegaly ≥ 5 cm and were evaluable for spleen response; 18 (44%) of these 41 experienced clinical improvement (CI) (IWG criteria). Three patients had CI in platelet count (IWG criteria), and one patient had CI in hemoglobin (IWG criteria). Twenty-one patients achieved stable disease (IWG criteria). Overall, 39 (70%) of the 56 MF patients experienced CI or stable disease. Among all enrolled patients, duration of progression-free survival (PFS) ranged from 1 to 875 days (median, 563 days), with an estimated 67% rate of PFS at 12 months (Kaplan-Meier). **Conclusions.** SB1518 shows promising efficacy in MF patients with splenomegaly. Once-daily dosing is well tolerated to 29 months, with manageable GI toxicity as the main AE. SB1518 does not appear to cause myelosuppression; patients with significantly impaired hematopoiesis can receive full-dose daily SB1518 without ex-acerbating hematocytopenias.
Pegylated interferon alpha-2a in patients with myeloproliferative neoplasms (MPN): International experience in 90 cases

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Background. Pegylated interferon alpha-2a (Peg INF2a) has been demonstrated to be active therapy for high risk essential thrombocythemia (ET) and polycythemia Vera (PV), as well as for treatment of early myelofibrosis (MF). We retrospectively analyzed the outcomes of Peg INF2a therapy in MPN patients treated outside the constraints of a clinical trial in the USA and EU. Methods. Clinical records of MPN patients treated at the participating centers, receiving Peg INF2a outside of the context of a clinical trial, were analyzed for response (ET and PV by ELN criteria, MF by EUNMET and IWG-MRT criteria), toxicity, and duration of response. Results. Patients: 90 patients were identified [46 PV (51%), 30 ET (33%), 14 MF (16%)] with a median age (57) and gender distribution (56% Females) typical for the disorders. The patients were a median of 96 months (2.0-324 months) after the diagnosis of the MPN and 65% harbored the JAK2-V617F mutation. 80% of patients had received at least one prior cytoreductive therapy for their disease (58 hydroxyurea, 21 anagrelide, 21 prior interferon (non pegylated)). There were 18 patients with vascular events (15 thrombotic, 3 hemorrhagic) prior to initiating Peg INF2a, with no vascular events occurring while on therapy. Therapy. Median starting dose of Peg INF2a was 90 µg/week (range: 45-180) with peak doses ranging from 45 to 270 µg. A total of 75 patients (83%) remain on Peg INF2a with median duration of treatment of 17 months (range: 2.0-58). Toxicity. Overall the Peg INF2a was well tolerated. Hematological toxicity was Gr 3 or lower. There were 6 cases with anemia (7%), 9 with thrombocytopenia (10%) and 9 had leukopenia (10%). Most common non-hematologic toxicities were fatigue Gr 1-3 in 17% (9%), 1 LFT elevation in 6% (7%) and 2-3 mood disorders in 3% (8%) patients. Only 11 (12%) discontinued therapy secondary to toxicity. Response. ET-PV. By ELN criteria, 20 PV patients achieved CR (43%), 21 achieved PR (46%) and NR in 5 (11%). In ET, the responses were CR in 15 (50%), PR in 10 (30%) and NR in 5 (15%). MF: the responses by IWG criteria were 1 CR (7%), 2 PR (14%), 4 CI (26%) and 7 SD (50%). By EUNMET, there were 2 CR (14%), 5 Major responses (36%), 4 moderate responses (29%), 1 minor response (7%) and 2 no response (14%). Conclusions. Peg INF2a used at doses consistent with published clinical trials is active and well tolerated when administered in an active clinical setting outside of the support of a clinical trial. Given the majority of patients had previously failed cytoreductive therapy these results further substantiate prior observation of Peg INF2a in MPNs. Upcoming randomized clinical trials through the Myeloproliferative Disorders Research Consortium will help further define the role of Peg INFα as first line therapy in high risk MPNs.

Sequential evaluation of chromosomal aberrations using high-resolution SNP microarrays in patients with myeloproliferative neoplasm experiencing disease progression

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Background. Myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Both PV and ET may evolve into myelofibrosis, and all conditions are also characterized by an increased risk of progression to acute myeloid leukemia (AML). The genetic mechanisms underlying these transformations are poorly defined at present. Aims. The aim of the present study was to establish whether disease progression is associated with development of chromosomal aberrations in MPN, and if the acquisition of these abnormalities has an impact on overall survival. Methods. This study included 24 MPN patients who progressed to secondary myelofibrosis (MF) or AML and could be followed with collection of at least two sequential DNA samples in different phases of disease. In detail, 14 patients progressed from chronic-phase MPN to AML, five patients from PV to post-PV MF and five from ET to post-ET MF. All patients provided their written informed consent before DNA collection. The Genome-Wide Human SNP 6.0 Array was used to detect chromosomal aberrations, including copy number variation (CNV) and copy-neutral loss of heterozygosity (CNOH). Paired samples collected before and after disease progression were compared by means of the Wilcoxon test for paired data. Results. Considering the whole cohort, disease progression from chronic-phase MPN to secondary MF or AML was associated with a significant increase in the number of chromosomal aberrations (P=0.0025) without any significant change in JAK2 (V617F) mutant allele burden (P=0.189). This increase remained statistically significant even after distinguishing patients who progressed from ET to post-ET MF and those with evolution from PV/ET to secondary MF (P=0.043). As abnormalities involving chromosome 5, 7 and 17p have been previously shown to be associated with worse survival in de novo AML, we evaluated whether the acquisition of these specific aberrations during follow-up had an impact on survival. By univariate analysis, the acquisition of one or more of the above aberrations was associated with reduced survival from the time of the initial diagnosis of MPN (Hazard ratio 18, 95% CI 1.9-164, P=0.011). Fisher exact test showed that the acquisition of aberrations of chromosome 5, 7 and 17p was closely associated with leukemic transformation (P=0.015). Conclusions. MPN patients who progress to secondary MF or AML show a significant increase in the number of chromosomal aberrations such as CNV and CNOH. The acquisition of aberrations involving chromosome 5, 7 and 17p is closely associated with leukemic transformation.

Management of elderly patients with essential thrombocythemia: An observational study of 471 patients of 80 years and older included in the EXELS European study

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Background. Essential thrombocythemia (ET) is usually diagnosed in patients aged around 60 years and its incidence increases with age. According to international guidelines, an age above 60 years is a risk factor for thrombosis and therefore, all patients aged ≥60 years receive cytoprotective therapy. However, there are no pharmaco-epidemiological data available in elderly ET patients. We identified patients aged ≥80 years in the cohort of ET patients included in the European observational EXELS (Evaluation of Xagrid Efficacy and Long-term Safety) study. Aims and Methods. To present data from elderly ET patients enrolled in this observational study. Clinical and biological data are collected over 12 months. Analyses were performed in Feb 2011 (based on data cut in Sept 2010). Results. Among the 3654 patients included from 13 European countries, 471 patients were ≥80 years at study enrollment. Across countries, the proportion of included patients aged ≥80 years ranged from 10 to 19%. There was no difference between patients aged ≥80 years (elderly) versus <80 years regarding gender (66% and 60% females, respectively), history of vascular events (41% vs. 36%), proportion of treatment-naïve patients (16% vs 20%), and antiaggregation therapy (both 69%). Anagrelide was the current cytoprotective treatment at enrollment in 40% of patients <60 years, 14% of patients 60-79 years, and 9% of patients ≥80 years. Evolution of blood cell count over time showed comparable changes in platelet counts in all patients, irrespective of age. Hematological tolerance to cytoprotective therapy was also comparable between elderly and younger patients. Switch from anagrelide to another cytoprotective therapy.
therapy was recorded in 10% of patients <80 years, and in 26% of those ≥80 years. The proportion of patients switching from hydroxyurea (HU) to another drug was similar in both age groups (10% and 12%, respectively). Median time to switch from anagrelide was 239 days in elderly patients, and 266 days in younger patients. Reasons for switch from anagrelide in patients ≥80 years was intolerance in 7/18 cases (vs 30/97 in patients <60 years), and no elderly patient changed because of inefficacy (15/97 for HU in <60 years). Reasons for switch from HU in patients ≥80 years was intolerance in 17/57 (vs 30/105 in patients <60) and inefficacy in 10/57 (vs 26/105 in patients <60). The number of patients with predefined events recorded (including vascular events and transformation to leukemia) was numerically higher in patients ≥80% of patients ≥80 vs 7% in patients <60, and 12% in patients 60-79 years), but numbers are too low to allow subgroup analyses. Conclusion. This cohort of 471 elderly patients (age ≥80 years) with ET enrolled in a prospective observational study shows that elderly patients' characteristics do not differ substantially from those of younger patients at presentation. These elderly patients received similar management across EU countries, and the use of anagrelide decreased along with increasing patient age. Anagrelide tolerability and efficacy appeared comparable in elderly and younger patients, and no new safety concerns arose in this patient population.

0912 RESULTS USING THE MODIFIED MYELOFIBROSIS SYMPTOM ASSESSMENT FORM (MFSAF V2.0) IN COMFORT-I: A RANDOMIZED, DOUBLE-BLIND PHASE III TRIAL OF JAK1/2 INHIBITOR RUXOLITINIB VS PLACEBO IN MYELOFIBROSIS (MF)

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Background. Symptom burden is a major component of MF. The MFSAF (Mesa et al. Leuk Res. 2009) was developed to measure MF-associated symptoms. The modified MFSAF v2.0 diary (a refined version of the instrument) was implemented to evaluate effects of the JAK1 and JAK2 inhibitor ruxolitinib (INC018424) on MF symptoms over a 24-week period compared with placebo in a randomized trial. Methods. Ruxolitinib Trial: 309 patients with a intermediate-2 risk MF provided informed consent and were randomized to start placebo or ruxolitinib at doses of 15 or 20 mg BID depending on baseline platelet count, with dose modifications allowed for efficacy and safety. Symptoms Change Assessments: The modified MFSAF v2.0 diary is a daily e-diary comprising 7 items, each scored on an 11-point scale from 0 (absent) to 10 (worst imaginable) in the 24 hours prior to assessment. The instrument measures patient-reported symptoms and impacts: abdominal discomfort (AD), abdominal fullness/early satiety (ES), bone pain (BP), pain under the left side of ribs (L), night sweats (NS), itching (I), and MF-related inactivity (first 6 items are pooled to create the total symptom score [TSS]). Patients completed the modified MFSAF v2.0 diary daily for 1 week prior to starting therapy (baseline, BL) and for 24 weeks on therapy. A Patient Global Impression of Change (PGIC) scale was used for patient self-evaluation of treatment benefit. PGIC was measured monthly from BL through week 24 using a 7-point scale (1-very much improved to 7-very much worse). Change from BL for individual symptom scores and the TSS were anchored to PGIC responses at week 24. Statistics: The proportion of patients achieving ≥50% improvement (ie, treatment responders) in individual symptom scores and TSS were compared (ruxolitinib vs placebo) using chi-square tests. Results. 148 patients receiving ruxolitinib and 152 receiving placebo completed diary entries. No significant difference existed in individual BL mean symptom scores between the two groups. Mean change for TSS from BL to week 24 for ruxolitinib was -8.6 (18.0 to 9.4) vs 3.2 (16.5 to 19.7) for placebo (p<0.0001). The individual response rates for symptom improvement were: AD, 53% vs 5.5%; BP, 45% vs 5.5%; ES, 45% vs 5.5%; NS, 46% vs 5.5%; and itchiness, 45% vs 5.5%. The proportion of patients achieving ruxolitinib vs placebo, respectively (p<0.0001). Using a moving 7-day average of TSS, the median time to response was 4.4 weeks for patients receiving ruxolitinib but could not be estimated for placebo because of insufficient responders over the 24-week period. At week 24, treatment responders for individual symptoms for ruxolitinib vs placebo were: AD, 48.3% vs 9.4%; ES, 48.3% vs 10.9%; BP, 39.6% vs 9.1%; NS, 46.9% vs 11.2%; I, 58.5% vs 11.5%; and inactivity, 53.1% vs 10.9% (all p<0.0001). 91.2% of ruxolitinib patients who were TSS responders had PGIC scores of “much” or “very much improved,” while 78.6% of placebo nonresponders had PGIC scores of “unchanged” or “worsened.” Conclusions. Serial administration of the modified MFSAF v2.0 diary demonstrated that treatment with ruxolitinib provided rapid, significant, and sustained improvements in MF symptoms vs placebo over the 24-week treatment period.

0913 SIDE EFFECTS OF HYDROXYUREA IN CLASSIC CHRONIC MYELOPROLIFERATIVE NEOPLASMS. A RETROSPECTIVE STUDY OF 3,411 PATIENTS

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Background. Hydroxyurea (HU) is the drug of choice for the treatment of patients with high risk myeloproliferative neoplasms (MPN). Its more frequent side effects are represented by gastrointestinal toxicity, cutaneous and mucosal toxicity, pulmonary toxicity and fever. A number of anecdotal cases have been reported in the literature, but epidemiological information on large series of patients are not yet available. Aims. To collect information on the rate and characteristics of HU-related effects, we examined data from a retrospective survey of ten hematological centers in Italy. To ensure consistency of data, we only included clinically relevant HU-related manifestations such cutaneous and mucosal lesions, fever and pneumonitis. Methods. This study was performed within the GlMEMA (Gruppo Italiano Malattie Ematologiche Maligne dell’Adul)to MPN Working party, under the auspices of AGIMM (AIRC-Gruppo Italiano Malattie Mileoproliferative). Centers were asked to identify, in their own data base, all subjects with a diagnosis of MPN who had received hydroxyurea and developed one of the side effects considered above. Diagnosis of PV, ET or PMF was made according to the WHO2000 or PVSG/WHO2001 criteria, while diagnostic of post-PV or post-ET MPF was made according to the IWG-MRT plus the histological WHO criteria. Furthermore, for comparison populations, each participating centre provided information on the total number of referring patients with matched diagnosis who had been in treatment with HU and who had not developed side effects in the same period of time. Results. The whole study population was comprised of 3,411 MPN patients, of whom 963 were PV, 1912 ET, 357 PMF, 93 PPV. During a mean period of time.

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days (range, 1-109) of treatment at median dosage of 0.5 g daily (range, 0.15-1 g), for a total HU median dose of 15 g (range, 0.5-52.5 g) for patient. Muco-cutaneous lesions were reported by 167 patients; 32 patients developed mucosal lesions, 118 patients presented cutaneous ulcers, while other cutaneous lesions including keratosis, dyschromia, basalioma and dermatitis developed in 21 patients, as detailed in the table. Two patients reported both mucosal and cutaneous lesions. Conclusions: With the intrinsic limitations of the retrospective design, this study provides, for the first time, an estimate of the rate of HU-related side effects (5%) overall strengthening the good tolerability of the drug.

0914
VALIDATION OF THE MYELOPROLIFERATIVE NEOPLASM SYMPTOM ASSESSMENT FORM (MPN-SAF) IN FRENCH, SPANISH, GERMAN, AND ENGLISH (UK)

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Background. We previously validated the MPN-SAF, a unique instrument for assessment of MPN symptoms based on patient reported outcomes, among 402 MPN patients including independent translations in English (USA), Italian and Swedish (Blood 2010). Aims. We sought to further validate MPN-SAF using prospective translations in French, Spanish, German, and Dutch and a new comparison cohort for English (UK). Methods. The MPN-SAF was translated using previously reported methodology involving four collaborating translators. MPN patients completed a symptom packet during a physician visit consisting of the MPN-SAF, EORTC-QLQC30, and survey-related feedback. Concurrently, physicians provided assessment of patient symptom burden and pertinent demographic and disease information. Results. Patients: 1,091 MPN-SAF surveys were administered (English (UK) (N=57), Dutch (N=236), French (N=482), German (N=59), and Spanish (N=197, including Argentina (n=22), Uruguay (n=8), Puerto Rico (n=10), and Spain (n=157)) in 433 ET (42%), 393 PV (38%), and 197 MF patients (19%) (8 missing). Participants were of typical age License 81.0, range 20-94 years) and gender (54% female) of disease. Prior hemorrhage (5%) and thrombosis (24%) were frequent, as were hematological abnormalities of anemia, thrombocytopenia, or leukopenia (21%). Validation of New Translations of the MPN-SAF: Patients were symptomatic (≥50% prevalence) in the majority of MPN-SAF items (Table 1). MF in general had the highest symptom burden and prevalence of symptoms, followed by PV and then ET. Fatigue was the most prevalent item (89%), with moderate or severe rating present in 60% of patients. Similarly, 75% of patients had reduced QOL and this reduction was moderate-to-severe in 34%. Patients rated the MPN-SAF as “easy to understand” (1.7/10) and having addressed most symptoms (2.0/10). Comparison to EORTC-QLQC30: Strong correlations existed between individual symptoms represented on both the MPN-SAF and the EORTC-QLQC30 including fatigue, inactivity, insomnia, and bone pain (r=0.51 to 0.82, all p<0.001). Additionally EORTC-QLQC30 subscales were highly correlated with corresponding items of fatigue, inactivity, concentration, and sad mood (r=0.50 to 0.75, all p<0.001). Comparison to Physician Perceptions: Physician’s blinded opinions of patients’ symptoms displayed strong correlations for fatigue and weight loss (r=0.5, p<0.001 for both), with moderate correlations for fevers, night sweats, bone pain, and pruritus (r=0.34 to 0.48, p<0.001). Comparison Across Languages: After adjusting for age and MPN type, there were no significant differences across languages for specific MPN-SAF items of abdominal pain, abdominal discomfort, headache, numbness, insomnia, fever and weight loss. There were no significant differences between UK and USA cohorts for any MPN-SAF item. Comparison with prior MPN-SAF: After adjusting for age and MPN type, there were no significant differences between current data and prior English (USA), Swedish, and Italian cohorts, except for early satiety, inactivity, headache, and QOL (p<0.05). Conclusions. The MPN-SAF is a valid PRO assessment of symptom burden for MPN patients worldwide. Strong correlations were seen between the translation and co-validation measures. Minor language-specific variations in MPN-SAF severity exist which likely represent cultural influences, language nuances, or variations in patient cohorts. Further use of the MPN-SAF in clinical trials is recommended as a tailored assessment of MPN symptom burden internationally.
**0915** SYMPTOMATIC BURDEN OF MYELOPROLIFERATIVE NEOPLASMS (MPN) IN FRANCE: A PROSPECTIVE TRIAL OF 482 PATIENTS

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**Aims.** We prospectively sought to gauge the cultural relevance of Myeloproliferative neoplasms (MPNs) are a subset of hematologic malignancies with significant symptomatic burden and a rapidly evolving treatment options. We have previously reported on the disease burden and impact on Quality of Life (QoL) among afflicted patients, but no prospective trials existed among a sizable European cohort. **Methods.** We prospectively sought to gauge the cultural relevance of a measure of symptom burden amongst a large cohort of MPN patients in France. **Results.** Patient completed a symptom assessment packet including a French translation of the Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF: French) and the French EORTC-QLQ-C30. Physicians concurrently completed a form detailing patient demographics, perception of the pt's symptomatic burden, and MPN diagnosis, treatment and clinical course. **Results.** Patients: 482 patients (ET (N=227; 48%), PV (N=175; 37%) and MF (N=75; 16%)) were prospectively enrolled from university and private hospitals in France. Patients were of a median age (63: range 22-91 years) and gender (56% females) typical of the disease with a median of 7 years (range:1-35 from their diagnosis. Patients frequently had a history of either thrombotic events (27%) and/or hemorrhagic events (4%). Anemia (13%), polycythemia (5%), leukopenia (5%), thrombocytosis (9%) and thrombocytopenia (9%) were common. Symptomatic Burden: The MPN-SAF: French indicated that insomnia, fatigue, problems with sexuality, numbness, and inactivity were most severe among French patients (see Table 1). Symptoms with highest prevalence included insomnia, numbness, early satiety, inactivity and sad mood. Similar to our prior studies, symptomatic burden was most severe and prevalent in MF, except for headache which was least severe in MF. Interestingly, pruritus was not most burdensome in PV patients. **Validation Analysis:** EORTC-QLQ-C30: Consistent with our experience findings, Pearson correlations between MPN-SAF: French individual symptom scores and the French EORTC-QLQ C30 showed excellent correlations with co-validation questions (p<0.001). Additionally, excellent correlations were demonstrated between EORTC-QLQ-C30 subscales and corresponding MPN-SAF measurements, particularly for items of BFI, inactivity, concentration, and sad mood (p<0.001). Physicians and Patient's perceptions: Correlations between physician's blinded perceptions of disease burden and patient-reported symptom severity in six categories of disease symptoms (night sweats, fevers, fatigue, weight loss, bone pain, and pruritus) were excellent for all items (p<0.001). Patients indicated that they felt that the survey was comprehensive of their disease symptoms (mean score 2.2/10) and easy to understand (mean score 1.7/10). Conclusions. The MPN-SAF: French is an easily administered, clear 27-item inventory of patient-reported outcomes that is specific to MPNs and validated by 1) comparison to compiled international data on MPN-SAF scores and 2) the correlation with the EORTC-French. Utilization of the instrument in French MPN clinical trials will serve as a valuable and specific clinical marker of disease symptom severity among this population.

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**Results.** Physicians concurrently completed a form detailing patient demographics, perception of the pt's symptomatic burden, and MPN diagnosis, treatment and clinical course.

**Table 1.**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>MPN-SAF</th>
<th>EORTC-QLQ-C30</th>
<th>Pearson Correlation</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>0.35</td>
<td>0.32</td>
<td>0.62</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Night sweats</td>
<td>0.36</td>
<td>0.31</td>
<td>0.63</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fevers</td>
<td>0.37</td>
<td>0.31</td>
<td>0.64</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.37</td>
<td>0.32</td>
<td>0.65</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Night sweats</td>
<td>0.38</td>
<td>0.33</td>
<td>0.66</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fevers</td>
<td>0.39</td>
<td>0.34</td>
<td>0.67</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fatigue</td>
<td>0.40</td>
<td>0.35</td>
<td>0.68</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Night sweats</td>
<td>0.41</td>
<td>0.36</td>
<td>0.69</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Fevers</td>
<td>0.42</td>
<td>0.37</td>
<td>0.70</td>
<td>p&lt;0.001</td>
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</tbody>
</table>

**Discussion.** Mastocytosis is a rare disease characterized by an abnormal proliferation and accumulation of mast cells (MC) in several organs and tissues such as skin, bone marrow, liver, gastrointestinal tract and lymphnodes. It is a clonal disease associated to a somatic mutation of the proto-oncogene c-kit (KIT). Clinical signs and symptoms of mastocytosis mainly depend on the liberation of chemical mediators produced by the mast cells. While the diagnosis of systemic mastocytosis requires the presence of multifocal dense mast cell infiltrates in one or more organs, the diagnosis of cutaneous mastocytosis is based on the identification of histopathological findings. **Background.** Mastocytosis is a rare disease characterized by an abnormal proliferation and accumulation of mast cells (MC) in several organs and tissues such as skin, bone marrow, liver, gastrointestinal tract and lymphnodes. It is a clonal disease associated to a somatic mutation of the proto-oncogene c-kit (KIT). Clinical signs and symptoms of mastocytosis mainly depend on the liberation of chemical mediators produced by the mast cells. While the diagnosis of systemic mastocytosis requires the presence of multifocal dense mast cell infiltrates in one or more organs, the diagnosis of cutaneous mastocytosis is based on the identification of histopathological findings.
multiple extra-cutaneous organs (mostly bone marrow, due to the origin of MCs), mastocytosis encompasses a wide range of clinical entities, extremely heterogeneous for clinical course and prognosis. Due to its heterogeneity, mastocytosis is a multidisciplinary pathology involving different specialists. Anims. The Italian Mastocytosis Registry was constituted in 2009 with the aim of collecting data about patients diagnosed with mastocytosis at a national level. The Registry, a tool for data collection at a national level. The Registry is an opportunity to carry out epidemiological studies aimed at estimating prevalence, incidence and geographical distribution of the disease. It will also provide support and direction of the research on mastocytosis in Italy. The on-line database is a useful tool for data collection at a national level. The Registry is an opportunity for the understanding of this disease, also in comparison to other diseases. It will finally allow specialists to investigate possible prognostic factors and provide a starting point for the research into ad hoc therapies.

### VALIDATED AND CANDIDATE THROMBOTIC RISK FACTORS IN ESSENTIAL THROMBOCYTHESIA: PRELIMINARY ANALYSIS OF THE REGISTRO ITALIANO TROMBOCITEMIA (RIT)

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Background. In essential thrombocythemia (ET), the validated risk factors for thrombosis at onset and during the follow up are represented by age over 60 y, and history of thrombosis. The thrombocythemia, constitutive abnormality in ET, is associated with both thrombotic and hematologic complications. JAK2 mutation and leukocytosis have been reported as associated with high rate of thrombosis. Anims. To evaluate in a large cohort of ET patients the potential thrombotic risk factors as JAK2 mutation, leukocytosis, and other clinical and biological parameters. Methods. A cohort of ET patients (PVSG or WHO criteria) of the Registro Italiano Trombocitemia (RIT), with information also on the bone marrow biopsy at diagnosis, were considered for this retrospective analysis. Results. A total of 977 patients, 387 males and 590 females, presented at diagnosis: median age 55 y, median PLT count 783 x 10^9/L, median WBC 4.13 x 10^9/L, median Hb 14.1 g/dL, median platelet counts in 189 cases (19.5%), median platelet counts in 49 cases (50%). The patients at high risk (age over 60 y and/or history of thrombosis) were 511 (52%). During the follow up (4088 pt-y), thrombotic events were reported in 35 patients (3.6%). The thrombotic events at onset of disease were significantly related to: age over 60 (p 0.001), male gender (p <0.05), lower grade of thrombocytosis (PLT < 783 x 10^9/L, p 0.01), higher grade of leukocytosis (WBC > 4.1 x 10^9/L, p 0.01), and JAK2 V617F mutation (p 0.06). No relationship was found with bone marrow fibrosis grade. The 977 patients were subdivided in four groups: lower thrombocytosis and higher leukocytosis (group 1: 202 pts); lower thrombocytosis and lower leukocytosis (group 2: 270 pts); higher thrombocytosis and higher leukocytosis (group 3: 272 pts); higher thrombocytosis and lower leukocytosis (group 4: 197 pts). In those patients, the rate of thrombosis at onset was: 26.7% in the group 1 (both risk factors); 24.1% in the group 2 (PLT risk factor); 20.2% in the group 3 (WBC risk factor); 7.6% in the group 4 (no risk factors). The rate of thrombosis in patients with one or two of these risk factors was significantly higher than in patients with no risk factors. The thrombotic events during the follow up are still object of analysis. Conclusions. In this cohort of ET patients the rate of thrombosis at onset of disease has been confirmed to be related to age over 60 y. Moreover, a significant relationship has been found with male gender, JAK2 mutation, WBC count over the median value (8.8 x 10^9/L), and PLT count below the median value (783 x 10^9/L).

### INFLUENCE OF 46/1 JAK2 HAPLOTYPE IN THE NATURAL EVOLUTION OF JAK2V617F ALLELE BURDEN IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Hospital del Mar, Barcelona, Spain

Background. The 46/1 JAK2 haplotype predisposes to the development of JAKV617F-associated myeloproliferative neoplasms (MPN) but its clinical relevance after diagnosis is unknown. Objective. To assess the influence of 46/1 JAK2 haplotype on the natural evolution of JAK2V617F allele burden in patients diagnosed with JAK2V617F-associated MPN. Methods. JAK2V617F allele burden was prospectively measured in 62 patients with newly diagnosed JAK2V617F-associated MPN, corresponding to polycythemia vera (PV) and essential thrombocythemia (ET) in 26 and 36 cases, respectively. Molecular monitoring was performed at diagnosis and every 6-12 months while patients remained in follow-up in 44 patients whereas 18 patients showed a JAK2V617F increase higher than 10%. The mean increase in JAK2V617F allele burden from diagnosis to last sample was 4.5%, 6% and 15% in patients negative, heterozygous and homozygous for the 46/1 JAK2 haplotype, respectively (p<0.00001). When analyzing the 81 patients for which the percentage of bone marrow substitution was reported, there was a significantly positive correlation with the level of thrombocythemia (Spearman rank correlation 0.59; p<0.000001). Conclusions. This is the first spontaneous observational study on mastocytosis in Italy. The on-line database is a useful tool for data collection at a national level. The Registry is an opportunity for the understanding of this disease, also in comparison to other diseases. It will finally allow specialists to investigate possible prognostic factors and provide a starting point for the research into ad hoc therapies.

### BIOMARKERS ON OUTCOME OF PATIENTS WITH PRIMARY MYELOFIBROSIS

THE IMPACT OF CLINICO-HEMATOLOGICAL FEATURES AND MOLECULAR MARKERS ON OUTCOME OF PATIENTS WITH PRIMARY MYELOFIBROSIS

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Background. Primary myelofibrosis (PMF) is the rarest subtype of the Philadelphia-negative myeloproliferative neoplasms (MPN). Several
biomarkers, including Endogenous Erythroid Colony (EEC), PRV-1 mRNA expression, X-chromosome inactivation patterns (XCIIPs) in females, JAK2V617F and MPL gene mutations have been described as useful tools to characterize MPN. The implication of these biomarkers and their correlations to the clinico-hematological features and outcome remained to be determined in patients with PMF. Aims: We aimed to determine (1) the frequencies of the biomarkers, and (2) the correlations among the biomarkers, clinico-hematological data and outcome of PMF patients. Methods: Granulocytes were isolated from 115 patients with PMF diagnosed by WHO criteria. Allele-specific PCR assay was used to detect JAK2V617F, PCR followed by direct sequencing for MPL mutation. EEC assay was performed in a serum-free culture system. PRV-1 overexpression in granulocytes was assayed by RT-PCR TaqMan assay, and HUMARA-PCR assay for XCIIPs was performed in female patients. Results: Of the 115 patients, the median age was 63.8 years, 59 were males. The median level of Hb was 9.6 g/dL, WBC was 11.6 x 10^3/L and PLT count was 368 x 10^9/L. JAK2V617F was detected in 58 of 115 (50.4%) with homozygous pattern in 9, MPL mutations in 5 of 93 (5.2%). EEC formation was present in 42 of 85 (49.4%), and PRV-1 overexpression in 52 of 59 (54.2%). Clonal XCIIPs was detected in 19 of 22 female examined (86.4%). JAK2V617F mutation was strongly associated with the presence of EEC formation and PRV-1 overexpression (p<0.0001). EEC formation was highly associated with PRV-1 overexpression (p=0.0007) and lower grade bone marrow fibrosis (p=0.0245). Both JAK2V617F and EEC formation were associated with WBC =25.0 x 10^9/L and less circulating blast cells (<1%). PRV-1 overexpression was associated with a high WBC count only. Median overall survival (OS) was 70.2±11.3 months. Fourteen patients (12.2%) developed acute leukemia transformation. Adverse prognostic factors of OS were older age (> 65), Hb <10 g/dL, PLT count <100 x 10^9/L and absence of EEC formation in a univariate analysis; older age and lower Hb level in the multivariate analysis. Patients who had EEC formation and less circulating blast cells (<1%) at diagnosis had a longer time to leukemia transformation. JAK2V617F mutation and PRV-1 overexpression did not influence OS or time to leukemia transformation. Splenic radiation and ≥1% of circulating blast cells at diagnosis increased the risk of acute leukemia transformation, but other clinico-hematological data, the marrow fibrosis grade or the status of biomarkers did not. Conclusions: Our data showed that JAK2V617F was highly associated with EEC formation and PRV-1 overexpression. Factors that adversely affected OS included older age, lower Hb, lower PLT count and absence of EEC formation. Splenic radiation and higher circulating blast cells increased the risk of acute leukemia transformation.

This study was supported by grants DOH100-TD-C-111-006, BMRPG380031 and CMRPG330303.

0920 NEUROLOGICAL SYMPTOMS IN ESSENTIAL THROMBOCYTHAEMIA (ET): INCIDENCE, ROLE OF BRAIN IMAGING, INFLUENCE OF JAK2V617F

MUTATION, AND OUTCOME
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Background: ET is characterized by thrombotic and ischemic complications in various vascular territories, including the brain. Such neurologic complications include transient ischemic attacks (TIA), strokes or cerebral venous thromboses (CVT). In addition, patients may present with a broad range of transient neurological symptoms such as blurred vision, headache, tinnitus, dizziness. These subjective symptoms are usually considered to be due to cerebral ischemia and highly sensitive to low-dose aspirin (ASA). They were frequently reported in retrospective series (in 20 to 55% of pts), but their incidence in newly diagnosed patients was not clearly assessed prospectively. Patients and Methods: From January 08 to June 09, all consecutive newly diagnosed ET patients (WHO criteria) from our center presenting with neurological symptoms were included older age, lower Hb, lower PLT count and absence of EEC formation in a univariate analysis; older age and lower Hb level in the multivariate analysis. Patients who had EEC formation and less circulating blast cells (<1%) at diagnosis had a longer time to leukemia transformation. JAK2V617F mutation and PRV-1 overexpression did not influence OS or time to leukemia transformation. Splenic radiation and ≥1% of circulating blast cells at diagnosis increased the risk of acute leukemia transformation, but other clinico-hematological data, the marrow fibrosis grade or the status of biomarkers did not. Conclusions: Our data showed that JAK2V617F was highly associated with EEC formation and PRV-1 overexpression. Factors that adversely affected OS included older age, lower Hb, lower PLT count and absence of EEC formation. Splenic radiation and higher circulating blast cells increased the risk of acute leukemia transformation.

This study was supported by grants DOH100-TD-C-111-006, BMRPG380031 and CMRPG330303.

0921 PRELIMINARY DATA ON CLINICAL ASPIRIN RESISTANCE IN A LARGE COHORT OF PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background: Thrombotic complications are major cause of morbidity and mortality in patients with myeloproliferative neoplasm (MPN). Low-dose aspirin significantly reduce the risk of thrombotic complications in polycythemia vera (PV) patients, and is commonly used also in essential thrombocythemia (ET). In general population, however, some patients taking aspirin (ASA) have thrombotic event in spite of treatment: this is defined as "clinical resistance to ASA". The aim of our study was to retrospectively evaluate clinical ASA resistance in patients with MPN. Patients and Methods: The study comprehends 290 MPN patients diagnosed between 1970 and 2009 in accordance with the criteria in use at the time of the first observation and treated in agreement with the current treatment guide-lines. All major thrombotic events occurred during follow up were recorded. Among the 290 patients enrolled, 231 (group A; 79.6%) used ASA (100mg/day) and 59 (group B; 20.4%) did not. The main features of our patients are summarized in table 1.

Results: 16 patients (4 males, 12 females; 8 ET and 8 PV) of group A (6.9%) had a thrombotic event after a median disease duration of 6.04 years: 8 arterial thromboses (5.4% of ASA-treated patients) (4 coronary disease, 1 stroke, 2 TIA and 1 intestinal infarct) and 8 venous thromboses (5 deep and 3 splanchic vein thrombosis). 62.5% of patients had thrombosis after more than 5 years of treatment. All but 13 patients were JAK2V617F 10 females (6 ET and 4 PV, all carrying JAK2V617F mutation) of group B (16.9%), had a thrombotic event after a median disease duration of 4.19 years: 3 arterial (1 coronary disease, 1 stroke and 1 TIA) e 7 venous (3 deep, 3 splanchnic vein e 1 cerebral sinus thrombosis). The overall occurrence of thrombotic complications was significantly less (p = 0.03) in group A than in group B. Discussion. The concept of theraeutic resistance emerged in general population with the clinical obser-

Table 1.
viation that aspirin-takers are not invariably protected from acute cardio-
vascular events. Moreover, loss of therapeutic advantage has been found
in individuals on long-term treatment. Our study shows that also in
MPN patients ASA-resistance occurs, mainly in long-time treated pa-
tients. While it is not surprising that vein thrombosis are not prevented
by aspirin, the occurrence of arterial thrombosis in 3.4% of MPN treated
with ASA suggest that clinical ASA-resistance is not absent in this par-
ticular set of patients. No relation with JAK2 mutational status was ob-
served. Our results need to be confirmed by specific laboratory tests.

**0922**

**HAEMATOLOGICAL ABNORMALITIES ARE COMMON IN NEONATES WITH DOWN SYNDROME AND MAY REVEAL CLINICALLY ‘SILENT’ LEUKAEMIA**

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Background. Neonates with Down syndrome (DS) are uniquely predis-
posed to Transient Abnormal Myeloipoiesis (TAM), a leukemic disorder characterised by mutations in the GATA1 gene, which transforms
to a clonally-related myeloid leukaemia (ML-DS) in 20-30% of cases. Children with ML-DS often have no history of TAM, suggesting TAM can be ‘silent’ since full blood counts (FBC) and blood smears are not routinely
performed despite reports of frequent haematological abnormalities in DS neonates. Our preliminary results. The prospective Oxford Down Syndrome Cohort Study (ODSCS) systematically determined haematological indices and blood cell morphology in the first week of life in neonates with DS (n=170) compared to normal neonates (n=123). Mutational analysis of GATA1 was performed on leucocyte DNA by PCR. Results. Compared to gestation-matched controls, neonates with DS had higher Hb concentra-
tions (201±4 v 166±6 g/dL; p<0.0001), increased erythroblastosis (25.5±5 v 3.2±0.5/100 leucocytes; p=0.0001) and macrocytosis (MCV 107.6 v 101.5; p<0.0001). Neonates with DS had lower platelet counts (165±7 v 252±5×109/L; p=0.0001), 95.0% had abnormal platelet mor-
phology and almost half (71/170, 41.8%) were thrombocytopenic (platelets<150×109/L). Leucocytes (p=0.0012), neutrophils (p=0.0005), monocytes (p=0.0001) and basophils (p=0.0001) were higher in DS neonates and dysplastic features, rare in controls, were seen in all DS neonates. Fourteen neonates (7.5%) had a final diagnosis of TAM confirmed by the presence of exons 2/3 GATA1 mutations. Neonates with TAM had lower Hb concentrations (177.7±0.8 v 201±4.2 g/dL; p=0.0012) and higher MCV (113.3±2.8; p=0.0008) than DS neonates without TAM and a significantly higher mean platelet count (229.8±83.3 x 109/L; p=0.0042) with a very wide range (36-1208 x 109/L). Total leucocyte counts (383.5±3.4 v 152±0.6; p=0.0001) and neutrophils (16.2±2.2 v 9.7±0.5; p=0.0008) were higher in neonates with TAM compared to DS neonates without TAM but there was considerable overlap in values. Blast cells were present on blood smears from all neonates with TAM (33.3±4.5%, range 15-77%) and 98.1% of DS neonates without TAM (4.5±0.3%, range 0.2-18%) although only 7 neonates without TAM (4.4%) had >10% blasts and none of these neonates had GATA1 mutations. Au-
tomated blast cell counts were unreliable in nearly all cases. TAM was clinical-
ly ‘silent’ in 2/14 cases (14.3%) and there was no difference between the haematological features of these 2 neonates compared to the other 12 neonates with TAM. Conclusions. All neonates with DS have haematolog-
ical abnormalities usually affecting red cell, platelet and myeloid cell line-
age. The most serious complication, TAM, may be clinically silent. These data suggest that FBC and blood smears should be part of the assessment of all newborn babies with DS in the first week of life and screening for GATA1 gene mutations performed where blasts exceed 10%.

**0923**

**RISK OF EVOLUTION TO MYELOFIBROSIS AND ACUTE LEUKAEMIA IN PATIENTS WITH ET OR PV WITHOUT CLINICAL HEMATOLOGICAL RESPONSE. ACCORDING EUROPEAN LEUKAEMIA NET CRITERIA**

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Background. Chronic myeloproliferative diseases, Polycythemia vera (PV) and Essential Thrombocythaemia (ET) have the risk of evolution to Myelofibrosis (MF) and Acute Leukemia (AL). European Leukemia Net (ELN) have defined new criteria to asses clinical, histological and molecular response in order to evaluate prognosis and new therapeutic agents. Aims. We performed a retrospective analysis of outcome of PV and ET patients in order to analyzed incidence of transformation to MF or LA in relation to ELN clinical response criteria. Material and Methods. We collected data from clinical and laboratory data of 874 patients diagnosed of ET or PV and followed in a teaching hospital of Madrid during the last 20 years were analyzed. Results. 108 patients present ET at di-
agnosis and 38 PV. Mean age at diagnosis was 61 and 67 years respec-
tively. Incidence of ET was more often on females (57%/53%/42%/M) and PV was slightly more often on males (46%/54%/54%/M). A total of 68% develop MF or LA under evolution. ET develop MF in 6 patients (5%) and acute leukaemia (AL) in 2 patient (2,8%), 3 patients with PV develop AL (5,1%). Evolution appear at different time intervals: AL ap-
pear in patients between 6 and 17 years from diagnosis. MF was devel-
oped between 3 and 20 years (mean 14 years). Other 12 patients (8%) died from additional complications (2 solid neoplasia, 2 sepsis, 5 ETE 1 Hemorrhagic, 2 unknown). All were patients of high risk and none of those patients showed Complete Clinical Response according to ELN during follow up. 21 present major complications (thrombotic or hem-
orhagic) at diagnosis/or under evolution. Three of 6 patients with MF are still alive but present clinical resistance to HU before transforma-
tion. Conclusion. Resistance to HU appears as a bad prognosis in di-
dicator in patients with ET and PV. Treatment oriented to achieve com-
plete clinical response according to ELN seen to be mandatory in these patient population.

**0924**

**PROSPECTIVE VALIDATION OF THE DUTCH MYELOPROLIFERATIVE NEOPASM SYMPTOM ASSESSMENT FORM (MPN-SAF: DUTCH) IN 236 MPN PATIENTS**

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Background. Myeloproliferative Neoplasma (MPN) give rise to specific symptoms generally not assessed by validated Quality-of-Life (QoL) Questionnaires. In view of the reported high impact of these specific symptoms on QoL and social participation, detailed information on the effect of new developed therapies is of importance. The MPN-Symptom Assessment Form (MPN-SAF) is a concise instrument of pa-
tient reported outcomes designed to assess the unique spectrum of symptoms present in patients with MPN. Aims. We sought to validate this instrument and to assess symptoms in a representative Dutch pop-
ulation of patients with myelofibrosis (MF), essential thrombo-
cythaemia (ET) and polycythemia vera (PV). Methods. The MPN-SAF Dutch individual symptom scores and the Dutch EORTC-
QLQ-C30 were translated in Dutch by one of the authors fluent in both languages, confirmed by one member of the Dutch patient MPN Foundation and sent to all members of the Dutch MPN Foundation. The Dutch EORTC-QLQ-C30 was co-administered for validation purposes. In ad-
dition, data were compared to a cohort of 102 USA MPN patients com-
pleting the MPN-SAF English. Results. The questionnaires were sent to 374 patients. The response rate was 39%. A total of 236 patients were included in the analysis (ET [n=72; 30.5%], PV [n=119; 50.4%] and MF [n=45; 19.1%]), as 104 questionnaires (34%) had not been completely ple-
ting the MPN-SAF English. Comparison with the MPN-SAF English Pearson correlations between specific symptoms generally not assessed by validated Quality-of-Life (QoL) Questionnaires. Comparison with the MPN-SAF English Pearson correlations between specific symptoms generally not assessed by validated Quality-of-Life (QoL) Questionnaires. Comparison with the MPN-SAF English. Comparison with the MPN-SAF English. Comparison with the MPN-SAF English. Comparison with the MPN-SAF English. Comparison with the MPN-SAF English.
Conclusions. The MPN-SAF Dutch is a 19 item inventory of patient reported outcomes that is specific to MPNs. Additionally, the instrument is validated by 1) comparison to the Dutch EORTC-QLQ C30 2) the correlation with the MPN-SAF English. Utilization of the MPN-SAF Dutch will provide information on the effect of new treatment modalities on MPN-related symptoms. It will allow for useful comparison to patients in other countries and can be used in future international clinical trials. 34% of patients did not totally complete the questionnaire, however more detailed instructions will probably improve the use in general practice.

Background. The efficacy and safety of anagrelide (ANA) have been demonstrated in adults with essential thrombocytemia (ET). The number of children treated with ANA is very low and data on the long-term use of this drug are sporadic. Aim. This single-center study was aimed at evaluating the efficacy of ANA in children and adolescents with Philadelphia-negative myeloproliferative diseases (Ph- MPD), previously untreated or resistant/intolerant to other cytoreductive drugs. Methods. Patients (pts) aged <20 years (yrs) at the time of a Ph- MPD diagnosis were treated with ANA at a dosage ranging between 0.5-1.5 mg/day. An informed consent was obtained in all cases. All pts were also investigated for biological markers (JAK2 mutations, PRV-1 expression, thrombopoietin and its receptor (c-MPL) mutations, clonality on female pts). Results. From April 1997 to January 2007, 12 pts with ET (11 pts) or familial thrombocytosis (FT = 1 pt) (8 female, 4 male; median age at diagnosis: 10 yrs [range:5-19]; median age at the ANA treatment: 156/12 yrs [range: 5-31]) were treated with ANA alone (5 pts) or in combination with hydroxyurea (HU) when the platelet (plt) count was >1000 x 10^9 /L and symptoms were present (7 pts). Ten pts had previously received HU + pipobroman (2 pts) + interferon-α (1 pt) for a median time of 54/12 yrs; 2 pts were untreated. The median time from initial diagnosis was 54/12 yrs (range: 1 month - 11 yrs); the median plt count prior to treatment was 1062 x 10^9 /L (range 322-3338 x 10^9 /L). A complete response (plt <450 x 10^9 /L for >1 month) was recorded in 6/12 pts (50%) after a median time of 6 weeks (range 3-24 weeks) and a partial response (plt 450 - 600 x 10^9 /L for >1 month) was observed in 3 pts after 12 months, so that 9/12 (75%) pts achieved a response at a median time of 2 months after starting treatment. Interestingly, the dose of ANA was tapered and finally stopped in 2 complete responders after 32 and 72 months, respectively, of therapy and they show a normal plt count after 26 and 10 months, respectively, without no further treatment. Six of the 9 responders are still being treated after a median of 76/12 yrs. Ten pts experienced early isolated or combined side effects, as headache (n=7), abdominal pain (n=3), vomiting (n=1), diarrhea (n=1), that disappeared over time in all but 3 pts. In these cases, persistent headache resulted in discontinuation of ANA after 5 weeks, 11 and 28 months, respectively, of treatment. Over the long term, mild self-limiting anemia has been recorded in 2 females. None of the pts developed leukemia or myelofibrosis or thrombotic complications during the long-term treatment with ANA. Conclusions. In our experience, ANA proved effective in controlling the plt number in thrombocytemic pts. A decrease of side effects was observed during long-term treatment with ANA, although 25 % of the total cohort stopped ANA.

Table 1.
Non-Hodgkin lymphoma - Clinical 2

0926
A PHASE II TRIAL ADDRESSING THE ROLE OF HELICOBACTER PYLORI-ERADICATING ANTIBIOTIC THERAPY AS EXCLUSIVE TREATMENT FOR STAGE I-II1 LARGE B-CELL LYMPHOMA (DLBCL) OF THE STOMACH (HGL-1 TRIAL)

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Background. Helicobacter pylori (Hp) infection is associated with both marginal zone lymphoma of Mucosa-Associated Lymphoid Tissue (MALT)-type and DBLC of the stomach. Hp-eradication therapy is the standard treatment for limited-stage Hp-associated MALT lymphomas of the stomach, whereas the role of this strategy in gastric Hp-associated DLBCL is controversial, with successful reports in a few, small monoinstitutional retrospective studies. On this background, we conducted a multicentre phase II trial addressing the role of Hp-eradication therapy as exclusive treatment in gastric DLBCL. Aims. To assess feasibility, activity and efficacy of Hp-eradication therapy as exclusive treatment for limited-stage DLBCL of the stomach. Methods. Inclusion criteria were histopathological diagnosis of DLBCL, with or without concomitant MALT-type areas; Hp-infection assessed by breath test or on multiple gastric biopsies; stage I-II1 of disease; hemoglobin >9 g/dl; perigastric lymph nodes diameter <1.5 cm; normal LDH serum level. Registered patients received Clarithromycin 500 mg bid, tindazole 500 mg bid and omeprazole 20 mg bid, for 7 days as exclusive treatment. Objective response and bacterial eradication were assessed by gastric endoscopy-ultrasonography, biopsies and breath test after one and two months from antibiotics. Responsive patients were referred to observation; patients with stable (SD) or progressive (PD) disease received conventional treatment. Results. From 2003 to 2008, 15 patients (median age 70; range 40-77, 10 males) were registered. Five patients presented concomitants MALT areas, while 10 patients had de novo DLBCL. Five patients had stage IE, 10 patients had stage III disease. Five patients presented anemia; two patients had concomitant HCV infection. Eradication therapy was completed in all patients without relevant toxicity. Eradication was documented at one month in 14 patients and achieved after second-line antibiotic-therapy in the remaining patient. According to the WHO criteria, lymphoma regression was complete in 7 (47%) patients, partial in 3 (67%) with one SD and 4 PD. Objective response was not associated with stage or concomitant MALT areas. At a median follow-up of 37 months, six of the 7 CRs were relapse-free, the remainder experienced relapse at 10 months, with a median DFS of 31 months. Two of the three patients in PR achieved CR after rituximab. Patients with SD/PD after antibiotics achieved CR with salvage R-CHOP± radiotherapy; none of them experienced relapse after 6-93 months. No patient died of lymphoma; two patients died of cardiac failure and gallbladder cancer, respectively; the remaining 15 patients are alive (12 patients are disease-free), with a 5-yr OS of 92%. Conclusions. This is the first prospective trial addressing the role of Hp-eradication therapy as exclusive treatment in patients with gastric DLBCL diagnosed in Western countries. Patients with stage I-II1 DLBCL can be safely managed with this strategy. Half of treated patients will achieve long-term remission without chemotherapy, a critical issue considering that two-thirds of patients are >65 years old. Unresponsive patients can be safely salvaged with conventional treatment. Analysis of correlation between treatment efficacy and biological features of these lymphomas are advisable in order to select the best candidates for this ultraconservative approach.

0927
TEMSIROLIMUS FOR RELAPSED PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA: EFFICACY AND PHARMAKOKINETIC STUDY

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Background. Salvage treatment is poorly defined in primary CNS lymphoma (PCNSL). Temsirolimus is a selective inhibitor of cell prolifera-

Figure 1. OS by Kaplan Meier method of HSCT group and IPI.
OS were significantly better in patients who received HSCT, even in patients with unfavorable features compared with chemotherapy alone. Summary/Conclusion. All prognostic models were useful to evaluate the outcome of patients with PTCL and NKTCL, but IPI score did best in predicting OS.

This study also supported the role of HSCT in patients with high-risk or refractory PTCL or NKTCL.

0929

CLINICAL AND BIOLOGICAL DIFFERENCES BETWEEN PATIENTS WITH CHILDHOOD T-CELL LYMPHOBLASTIC LYMPHOMA (T-LBL) WITH AND WITHOUT MEDIASTINAL TUMORS

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Background. T-cell lymphoblastic lymphoma (T-LBL) is immature T-cell malignancies with similar morphological features and optimal treatment strategies with T-cell acute lymphoblastic leukemia (T-ALL). In adults, T-LBL is a rare form of non-Hodgkin lymphoma (NHL) with an incidence of < 1.7%. The incidence is the highest in children and adolescents, where T-LBL constitutes about 25-30% of all NHL. [Swedlow SH, et al., 2008]. Results of treatment with intensive ALL-like regimens are fine and significant adverse prognostic factors could not be identified due to the small number of patients (pts) with this rare disease. Aim of this study was to investigate the effect of individual clinical and biological features on the disease outcome. Methods. From May 1991 to October 2008, 58 pts (m-40, f-18) with de novo LBL were treated with NHL-BFM 90 or 95 protocols for non-B-NHL (ALL-type) and 6 (10%) - NHL-BFM 90 (NHL-type). In an analysis of prognostic factors in 48 pts with T-LBL treated with ALL-type therapy were included. Results. Median age at time of presentation was 11.0 (range 1.5-21.6) years. 45 (90%) patients have a T-cell immunophenotype. 53 (91%) had advanced (III, IV) stage. The presenting sites of T-LBL included mediastinal mass - 35 (78%) and bone marrow (BM) involvement - 13 (29%). The complete response (CR) rate was 94 and 83% for non-B-NHL and B-NHL treatment respectively. 5-years event free survival (5y-EFS) was 0.80 ± 0.06 (median of observation 4.1 years) and 0.67 ± 0.19 (5.1 years) respectively (p<0.05). 5-years overall survival (5y-OS) was 0.85 ± 0.05 and 0.80 ± 0.06 respectively (p<0.05). The clinical features and outcome the T-LBL pts treated with ALL-type therapy was demonstrated in table 1. In a situation without mediastinal tumors the prognostic role of 1.7 was unfavorable compared to without it (5y-EFS 0.58 ± 0.17 vs. 0.90 ± 0.05, p=0.036). Cases without mediastinal tumors were represented by early (pro-T/pre-T) immunological subtypes (100% vs. 36%, p=0.041) while those with mediastinal tumors by late (cortical/medullar T) subtypes. Sex, age, increased LDH, slow or fast therapy response, involvement of the central nervous system (CNS) or BM did not affect on the prognosis (p>0.05). Conclusions. Our data demonstrate that situation without the mediastinal tumors are a factor adverse prognosis for childhood T-LBL treated by ALL-BFM-type therapy. Based on the characteristics of a normal T-cell lymphopoiesis suggests the existence of significant biological differences between the variants of T-LBL with and without mediastinal tumors.

Table 1.

0930

IMPACT OF RITUXIMAB MAINTENANCE TYPE AND FCGRIIA AND FCGRIIIA GENOMIC PROFILE IN PATIENTS WITH PREVIOUSLY UNTREATED FOLLICULAR LYMPHOMA

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Background. The PRIMA study has demonstrated that maintenance with Rituximab (R) during two years significantly improve progression free survival in patients with previously untreated follicular lymphoma (FL) responding to immunochemotherapy. Polymorphisms in the IgG Fc receptor FCγRIIIA and FCγRIIIA genes have been associated with response in several lymphoma types. The aim was to retrospectively analyse our experience with R maintenance (RM) in pts with previously untreated follicular lymphoma (FL) responding to rituximab or immunochemotherapy and, also to analyse the impact of polymorphisms regarding progression free survival, incidence of hypogammaglobulinemia and Ig levels. Patients and Methods. patients with FL in CR or PR after first-line R or R-Chemo received RM: type 1) R 375 mg/m2/week x 4 consecutive weeks every 6 months during 2 years or type 2) R 375 mg/m2 every 6 or 2 months during 2 years. FCγRIIa and FCγRIIIa genotypes were determined following a PCR-restriction fragment length polymorphism (RFLP) method. Results. From May 1991 to October 2008, 58 pts were treated with NHL-BFM 90 or 95 protocols for non-B-NHL (ALL-type and RM1 22 (45%), 10 (20%) received antracyclin-containing chemo. Status previous starting RM: CR in 80% and PR in 20%. Type of RM: RM1 in 16 (41%) and RM2 23 (59%). The distribution of polymorphisms was: FCγRIIa HH 15 (22%), HR 2 (5%) and RR 22 (33%) and FCγRIIIa VV 9 (13%), VF 21 (34%) and FF 7 (18%). At a median follow-up since first R maintenance infusions of 40 months (3-106), 9 pts (23%) have relapsed and 4 (10%) have died. Overall and progression free survival at 4 years were 95% and 79%, respectively. Antracyclin-containing chemotherapy was significantly associated with a different probability of progression (HR 5.2; 95% CI 1.1-25.1, p=0.022), but not the following variables: status prior to maintenance, type of maintenance therapy. The two schedules of R maintenance were effective and all pts benefited independently of FCγRIIa and FCγRIIIa genotype. Levels of IgM diminished at the end of maintenance in pts with FCγRIIa HR-RR (p=0.019) and in those with FCγRIIIa VF-FF (p=0.017). IgG and IgA levels did not significantly change during maintenance. Conclusions. Maintenance for two years in patients with previously untreated follicular lymphoma (FL) responding to immunochemotherapy is a very active therapeutic strategy, but those pts receiving antracyclin-containing treatment had lower probability of progression. The two schedules of R maintenance were effective and all pts benefited independently of FCγRIIa and FCγRIIIa genomic profile. Levels of IgM were significantly influenced according to FCγRIIa and FCγRIIIa genotypes.

0931

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) AS A CONSOLIDATION OFFERS A DURABLE SURVIVAL BENEFIT IN PATIENTS WITH PERIPHERAL T-CELL LYMPHOMAS (PTCL)

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Background. Peripherial T-cell lymphomas comprise a heterogeneous group of malignancies which are characterized by an aggressive disease course and a poor clinical outcome after conventional chemotherapy with 20-25% of patients being alive after 5 years from treatment initiation. ASCT for relapsed and refractory patients with PTCL seems to be an effective salvage regimen with event-free survival exceeding 35%, however the risk of disease progression or relapse remains high. Material and Methods. In a single centre retrospective study, we analyzed the results of ASCT in 29 patients with advanced stage PTCL. Patients were proceeded to transplant after achieving first or subsequent complete remission (CR) or partial response (PR) after conventional chemotherapy. Results. Twenty nine patients (15 male and 14 female) at a median age at diagnosis of 45 years (range 20-66 years) were analyzed. The study cohort included 16 patients with ALK-negative or unknown anaplastic large T-cell lymphoma (ALCL) and 13 with peripheral T-cell lymphomas unspecified (PTCL-U). There were no significant difference in
demographic and clinical features between both cohorts except for tendency for higher platelet count in ALCL subgroup (p=0.06). The majority of patients in both subsets had advanced disease at diagnosis (III and IV clinical stages; 73%) and B symptoms (76%). International prognostic index (IPI) ≥2 was demonstrated in 10 PTCL-U and 6 ALCL patients. Induction chemotherapy consisted of a median of six CHOP (cyclophosphamide, BCNU, etoposide) for 18% and BEAM (BCNU, cytarabine, etoposide, melphalan) for 4 patients. The engraftment was observed in all transplanted patients, there were no significant difference in hospitalization time and number of infections between compared subgroups. Among 29 transplanted patients, 7 (24%) died due to disease progression. Twenty two patients remained in CR. The median follow-up time was 4 years. The 4-year probability of the overall survival for whole group was 57% (58% for PTCL-U and 48% for ALCL). The probability of disease-free survival (DFS) after 4 years was 55% for whole cohort (56% for PTCL-U and 44% for ALCL). In an univariate analysis older age, the higher number of prior lines of chemotherapy and disease status at transplant were found to be associated with the lower probability of survival, but only age >45 years was found to influence the outcome independently in multivariate analysis. 

Conclusions. We have confirmed that ASCT as consolidation therapy for PTCL is a safe and efficient procedure for PTCL patients who are chemotherapy and disease status at transplant was associated with inferior clinical outcome.

**0932**

ANAPLASTIC LARGE CELL LYMPHOMA ALK POSITIVE AND ALK NEGATIVE: CHARACTERISTICS AND OUTCOME OF 98 CONSECUTIVE PATIENTS FROM A SINGLE INSTITUTION OVER 25 YEARS

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Background. Anaplastic large-cell lymphoma (ALCL) is a T-cell lymphoma characterized by expression of CD 30, often associated with t(2;5) (p23;q35) involving anaplastic lymphoma kinase (ALK). ALK positive and ALK negative systemic ALCL are defined as distinct entities in the WHO 2008 classification. The first-line of treatment in adults patients is evaluated in clinical trials, especially in ALK negative cases.

Purpose. Retrospective evaluation of outcome in 98 patients with ALCL, ALK+ and ALK- according to clinical features and treatment regimen.

Patients and Methods. Between 1985 and 2004 majority of patients received CHOP or CHOP-like chemotherapy (Group 1, n=75). Since 2004 23 patients were treated with intensive chemotherapy and ASCT, the German Multicentre ALL Study Group B-ALL/NHL 2002 (Group 2, n=23). ALK protein expression was tested in 82 pts. Kaplan-Meier method was used to estimate overall survival time and progression free survival time. Cox proportional hazards model was used in multivariate analysis of clinical variables. Results. Median age (range) was 40 (18-83), 59% were male, 45% were in clinical stage (CS) IV, 61% had B symptoms, 46% had bulky disease, 50% had LDH > N, and 56% had IPI score >1. Group 1 and Group 2 were significantly different in age: 42 vs 33 (p=0.005), ALK positivity: 41% vs 82% (p=0.001), HGB>12 g/dl: 55% vs 75% (p=0.008), and CS IV: 39% vs 65% (p=0.002), respectively. Median overall (OS) and progression free survival (PFS) was 63,5 months, 95% C.I.= [0.130,9] and 31,2 months, 95% C.I.= [0.72,40], respectively. Five years OS and PFS was 50%, 95% C.I. = [40%, 61%] and 45%, 95% C.I. = [34%, 56%]. On multivariate analysis, CS IV (p=0.001), B symptoms (p=0.001), bulky disease (p=0.001) and LDH> N (p=0.005) were independent adverse factors for OS and PFS. Median number of all given cycles before ASCT for PTCL-U and ALCL was 8 (range 3-20) and 7 (3-18), respectively. There was no difference in median time from diagnosis to ASCT between subgroups. The disease status at diagnosis was as follows: eight CR and 5 PR for PTCL-U and 9 CR and 8 PR for PTCL-U and ALCL. Conditioning regimen before ASCT consisted of GV (cyclophosphamide, BCNU, etoposide) for 15% and BEAM (BCNU, cytarabine, etoposide, melphalan) for 4 patients. Kaplan-Meier analysis of clinical variables. 

Conclusions. We have confirmed that ASCT as consolidation therapy for PTCL is a safe and efficient procedure for PTCL patients who are older age at transplant was associated with inferior clinical outcome.

**0934**

FEVER NEUTROPNIA RISK ASSESSMENT AND GRANULOCYTE-COLONY STIMULATING FACTOR SUPPORT IN PATIENTS WITH FOLLICULAR LYMPHOMA RECEIVING R-CHOP-21

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Background. EORTC guidelines (2010) recommend granulocyte colony stimulating factor (G-CSF) primary prophylaxis (PP) for patients receiving chemotherapy at overall risk of febrile neutropenia (FN) ≥20%. CHOP ± rituximab (R) is frequently used in patients with high risk follicular lymphoma both at presentation and relapse. R-CHOP-21 regimen in combination with older age and other individual risk factors can lead to high FN risk, where G-CSF PP is recommended. Anns. Describe current clinical practice in FN risk-assessment and G-CSF use in follicular lymphoma patients receiving R-CHOP-21 chemotherapy.

Methods. IMPACT NHL is a multicentre, international observational study conducted in Europe and Australia. Eligible patients were age ≥18 years and planned to receive ≥3 cycles of CHOP±R. Patients provided written informed consent where required. This analysis reports data from follicular lymphoma patients who received R-CHOP-21. The pri-
mary outcome measure was the proportion of patients with an investig-
ator-assessed individual-patient risk of FN ≥20% per guidelines who received PP G-CSF (pegfilgrastim or daily G-CSF initiated within days 1-7 of cycle 1). Results. Of 1829 NHL patients enrolled between 2005 and 2008, 345 had follicular lymphoma. Of these, the great majority (n=310, 90%) received R-CHOP-21 and are the focus of this analysis. The mean age was 58 (SD 12) years, almost half (48%) had poor FLIPI score for advanced NHL, a history of comorbidities, and 85% were previously untreated. FN risk was assessed by investiga-
tors as ≥20% in 57% (n=177) of patients, but only 37% of these (n=66) received PP G-CSF and 16% (n=28) experienced FN. Of 132 patients with <20% FN risk, 9% (n=12) received PP and 8% (n=11) experienced FN. In total, 39 patients (15%) experienced 48 separate FN events. FN was most commonly managed by hospitalization (33/48 events), fol-
lowed by home care (7/48 events), and outpatient visits and no action taken (both 3/48 events). Furthermore, unplanned hospitalizations oc-
curred in 53 (17%) patients; the most common reasons were neu-
tropenia/FN (25/53 hospitalizations, 54%) and non-haematologic ad-
verse events (24/53 hospitalizations, 35%); the median length of stay was 4 nights (interquartile range 3 - 8). The planned dose of chemotherapy matched the standard dose for each agent in R-CHOP (excluding vincristine and prednisone) in the large majority of patients. Most patients achieved a clinical response at the end of treatment, irrespective of G-CSF use (62% complete response, 30% partial response). Pegfilgrastim PP was received by 17% of patients (n=52). Among pa-
tients receiving daily G-CSF PP (n=26, 8%), the mean (±SD) number of doses per cycle was 4.9 (1.3). Patients who received PP G-CSF were older (mean 63 vs 56 years), more likely to have a history of comorbid-
ties (56% vs 44%) and a poor FLIPI score (53% vs 40%) than those who did not. Summary. Conclusions. Follicular lymphoma patients treated with R-CHOP-21 may be at risk of FN and related complications. Older patients with a high-risk profile were more likely to receive PP G-CSF; however, inconsistency was observed between investigator FN risk-as-
seessment and G-CSF administration, with only 57% of high-risk pa-
tients administered G-CSF PP per guideline recommendations.

0935
DOSE SELECTION FOR PHASE III STUDIES OF THE MONOCLONAL ANTI-CD20 ANTIBODY OBINUTUZUMAB (GA101) - A RATIONAL APPROACH

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Background. Obinutuzumab (GA101) is the first type II, glycoengi-
nerated humanized monoclonal anti-CD20 antibody to enter clinical trials. Phase I results. Two phase I studies, BO20999 and BO21003 (Salles, ASH 2008, 2009; Sehn, ASH 2009), in patients with CD20+
NHL showed that GA101 at doses of 50-2000mg was well tolerated, with no dose limiting toxicities and promising efficacy. Pharmacoki-
etic (PK) data from both studies indicated increasing plasma levels of GA101 following doses ranging from 400/800mg to 1200/2000mg, consistent with modeling and simulation target saturation levels. In contrast to the pre-clinical models, no clear dose-response relationship could be established, possibly due to patient variability including vari-
ous tumor sizes, histologies and number of prior therapies. However, GA101 PK data, similarly to published rituximab data, indicated that patients with higher disease burden may have faster clearance of GA101 and consequently these patients may require higher doses to achieve target saturation. Phase II results. To determine the phase III dose, NHL patients in the phase II part of study BO20999, (indolent [n=40] and aggressive [n=40 (25 DBCL/15 MCL)], were randomised to receive GA101 at either a dose of 400mg for all infusions or 1600mg on Days 1 and 8 and 800mg for subsequent infusions. Results showed that GA101 was well tolerated at both dose levels and favored the 1600/2000mg dose level, in indolent NHL patients with an end of treat-
ment response (EOR) of 55% (including 50% CR) in refractory patients, compared to the 400mg dose group (EOR 17%). (Salles EHA, ASH 2010). Higher GA101 plasma concentrations were observed in the 1600/800mg group compared to the 400mg group with both main Cmax and Cmin values rapidly reaching steady state after cycle 2 at the 1600/2000mg dose level. Steady state PK at cycle 2 was observed for the 1600/2000mg dose in indolent and aggressive NHL patients (Mean Cmax and Cmin values at steady state were 500- 600ug/mL for indolent and 300- 400ug/mL for aggressive patients), with target satu-
ration incomplete at the 400mg dose level. Thus, early target concentra-
tions could be best achieved using a 1600mg loading dose on d1 and d8. Modeling and simulation indicated that the same might be achieved if the induction dose for all cycles was to be administered throughout a treatment course and one additional dose given at d15 of the first cycle. Moreover, comparison of the PK data obtained from study BO20999 with that from study BO21003 (where GA101 was administered weekly over 4 doses), indicates that higher GA101 plasma concentra-
tions are achieved earlier with the more intensive regimen, indicative of target saturation. Summary. Based upon all available clinical data, a dose of 1000mg (flat dose) was selected for phase III studies in lymph-
oma, with GA101 to be administered on days 1, 8 and 15 of the first cycle to rapidly achieve and maintain adequate exposure levels. Con-
clusions. GA101 monotherapy shows promising efficacy in heavily pre-
advanced NHL patients, including refractory patients. The phase III dose of 1000mg is currently being investigated in combination with various chemotherapeutic agents in first line, relapsed and refractory NHL.

0936
TOLERANCE OF R-CVP FOR FOLLICULAR LYMPHOMA IN AN UNSELECTED POPULATION AND IMPACT OF DOSE REDUCTION ON OUTCOME

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Background. 6-8 cycles of R-CVP is widely regarded as standard initial therapy for advanced follicular lymphoma (FLC) with studies demon-
strating high response rates and durable remissions. However, there is very little published data on tolerance in unselected patients or the impact of dose reduction on outcome. Aims. To establish the proportion of unselected patients in routine practice able to receive full dose R-CVP for FLCL, and the impact of dose reduction on response rate and dura-
tion. Methods. All patients with stage 3 or 4 FCL (excluding grade 3b) commencing therapy between 1st Sept 2005 and 31st March 2010 were identified at four centres with similar patient populations and supportive care protocols. Patients with prior chemo- or radiotherapy for a haema-
tological malignancy, or HIV were excluded. Progression free survival (PFS) was calculated from date of first chemotherapy. Receiving fewer than 6 cycles was considered multiple drug dose reduction unless treatment was abandoned for progressive disease (1 patient). Results. 88 patients were available for analysis. Two dose reductions were achieved, one per follow up, these patients were excluded from analysis. Of the remainder, 46 (52%) completed 6 cycles of full dose R-CVP (Rituxinax 375mg/m2, CYclophosphamide 750mg/m2, Vincristine 1.4mg/m2, [maximum 2mg], and prednisolone 100mg for 5 days) and 59 had some dose and/or cycle number reduction. 2 patients received maintenance rituximab and were excluded from progression free survival analysis. Cyclophosphamide dose was reduced in 21 (median 80% of full 6 cycle dose), vincristine in 28 (median 50% full dose), rituximab in 15 (median 83% full dose),10 patients had s5 cycles (median 4), 18 patients had 6 cycles with reduc-
tion in only a single agent per cycle (2 rituximab, 6 cyclophosphamide and 10 vincristine), the other 21 had fewer than 6 cycles and/or reduc-
tions in dose of multiple agents. The commonest reasons for dose redu-
tion were age (55%), neuropathy (20%) and poor tolerance (9%).There was no difference in FLIPI (median 2), ECOG performance status (me-
dian 1) or median age (64 vs 65years) between those receiving full dose or not or between those receiving reductions in single agents or in mul-
tiple drugs. Median follow up was 27 months. PFS, complete and partial response rates were determined for full dose (695 days, 37% and 63%), any dose reduction (705 days, 56%, 61%), single agent (760 days, 28%, 67%) and multiple agent (706 days, 42%, 52%) dose reduction. There were no statistically significant differences between these groups (Figure 1 shows full dose vs any dose reduction). Summary/Conclusions. Outside clinical trials, a substantial proportion (45%) of patients with stage 3 or 4 FCL are deemed unable to receive R-CVP at full dose. However, mod-
erate reduction in the doses of its components has no significant impact
PET-SCAN FOR RESPONSE ASSESSMENT AFTER RITUXIMAB-CHOP (R-CHOP) IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMLBCL): PROGNOSTIC SIGNIFICANCE AND IMPLICATIONS FOR SUBSEQUENT RADIOTHERAPY (RT)


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Background. Burkitt lymphoma (BL) is the most aggressive B-cell lymphoma neoplasm, whose growth fraction approximates 100%, with specific chromosomal abnormalities (t(8;14)(q24;q32), rarely t(2;8)(p12;q32), (8;22)(q24;q11)). Despite the rapid proliferative rate, BL is one of the most chemosensitive lymphoid neoplasm. Though it was clear, that high intensive short-term alternating multiagent chemotherapy regimens are most effective in patients with BL, adults have a less favorable outcome than pediatric patients with BL. Aims: to evaluate the efficacy and toxicity of the protocol BL-M-04 for adult patients with BL. Methods: 46 previously untreated patients with BL were eligible for our study (they had specific c-myc rearrangement. All the patients (32 males and 14 females, mean age 29 years (from 15 to 62 years) participated in the study performed in the Russian Hematological Research Center between August 2003 and December 2010. The treatment was based on experimental high intensive protocol BL-M-04. BL staging criteria developed by S. B. Murphy were used to stage the patients. Stage I, II, III, IV were diagnosed in 3, 5, 16, 6 patients respectively. B-acute lymphoblastic leukemia (L3) was diagnosed in 16 (25%) patients. The main aim of the new treatment regimen was greater efficacy of therapy due to intensification and shorter treatment duration. The new treatment protocol is based on the modified NHL-BFM protocol for high risk patients with a reduced dose of methotrexate from 5000 mg/m2 to 1500 mg/m2. As BL is a chemosensitive tumor that often regresses after 1-2 courses of chemotherapy, we decided to treat patients with BL in 4 courses of chemotherapy (2 induction and 2 consolidation) irrespective of the initial tumor mass. As BL is most sensitive to high dose methotrexate and cytarabine, we used these drugs in the induction phase to achieve to maximize the cytoreductive effect. Courses A and C were used to achieve remission. Doxorubicin was added to course A, and methotrexate to course C. Consolidation courses were similar to induction courses. Hence, we used A and C courses (without course B), intensified with course B drugs (doxorubicin and methotrexate), the interval between the courses being 21 days. Results. 41 patients (89%) achieved a complete remission (CR) after 1-2 courses (18 patients - after the 1st course, 23 - after the 2d). 39 patients had persistent positive findings at repeated examination after RT, but only 2 of them indeed had active disease or relapsed later. Conclusions/Discussion. PET-scan remains positive in a substantial proportion of PMLBCL patients who achieve a radiographic response with R-CHOP. In the majority of them, however, 18-FDG uptake is relatively low. Persistence of a positive PET was not clearly associated with inferior outcome, when additional RT was administered, although higher SUVmax values might predict a higher risk of relapse. Among 13 non-irradiated, PET-neg patients, only 1 relapse would be preventable by RT. According to these data, patients with PMLBCL should not be forwarded to autologous transplant simply based on a positive post-R-CHOP PET-scan, if radiographic response is adequate.

Efficacy and Toxicity of a New Short-term High Intensive Protocol BL-M-04 for Adult Patients with Burkitt Lymphoma

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BACKGROUND. Burkitt lymphoma (BL) is the most aggressive B-cell lymphoma neoplasm, whose growth fraction approximates 100%, with specific chromosomal abnormalities (t(8;14)(q24;q32), rarely t(2;8)(p12;q32), (8;22)(q24;q11)). Despite the rapid proliferative rate, BL is one of the most chemosensitive lymphoid neoplasm. Though it was clear, that high intensive short-term alternating multiagent chemotherapy regimens are most effective in patients with BL, adults have a less favorable outcome than pediatric patients with BL. Aims: to evaluate the efficacy and toxicity of the protocol BL-M-04 for adult patients with BL. Methods: 46 previously untreated patients with BL were eligible for our study (they had specific c-myc rearrangement. All the patients (32 males and 14 females, mean age 29 years (from 15 to 62 years) participated in the study performed in the Russian Hematological Research Center between August 2003 and December 2010. The treatment was based on experimental high intensive protocol BL-M-04. BL staging criteria developed by S. B. Murphy were used to stage the patients. Stage I, II, III, IV were diagnosed in 3, 5, 16, 6 patients respectively. B-acute lymphoblastic leukemia (L3) was diagnosed in 16 (25%) patients. The main aim of the new treatment regimen was greater efficacy of therapy due to intensification and shorter treatment duration. The new treatment protocol is based on the modified NHL-BFM protocol for high risk patients with a reduced dose of methotrexate from 5000 mg/m2 to 1500 mg/m2. As BL is a chemosensitive tumor that often regresses after 1-2 courses of chemotherapy, we decided to treat patients with BL in 4 courses of chemotherapy (2 induction and 2 consolidation) irrespective of the initial tumor mass. As BL is most sensitive to high dose methotrexate and cytarabine, we used these drugs in the induction phase to achieve to maximize the cytoreductive effect. Courses A and C were used to achieve remission. Doxorubicin was added to course A, and methotrexate to course C. Consolidation courses were similar to induction courses. Hence, we used A and C courses (without course B), intensified with course B drugs (doxorubicin and methotrexate), the interval between the courses being 21 days. Results. 41 patients (89%) achieved a complete remission (CR) after 1-2 courses (18 patients - after the 1st course, 23 - after the 2d). 39 patients had persistent positive findings at repeated examination after RT, but only 2 of them indeed had active disease or relapsed later. Conclusions/Discussion. PET-scan remains positive in a substantial proportion of PMLBCL patients who achieve a radiographic response with R-CHOP. In the majority of them, however, 18-FDG uptake is relatively low. Persistence of a positive PET was not clearly associated with inferior outcome, when additional RT was administered, although higher SUVmax values might predict a higher risk of relapse. Among 13 non-irradiated, PET-neg patients, only 1 relapse would be preventable by RT. According to these data, patients with PMLBCL should not be forwarded to autologous transplant simply based on a positive post-R-CHOP PET-scan, if radiographic response is adequate.
this protocol can achieve rapid tumor regression with a short treatment duration due to chemotherapy intensification and acceptable toxicity.

**0939**

**IMPROVED OVERALL SURVIVAL FOR VERY ELDERLY PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA AFTER THE INTRODUCTION OF IMMUNOCHEMOTHERAPY**

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**Background.** Diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma subtype and, as in most other malignant diseases, the incidence is strongly related to increasing age. Population-based studies have reported that the median age for DLBCL patients is 70 to 72 years and as high as about 20 % of all patients are above the age of 80. The prognosis of DLBCL patients has improved considerably during the last decade, mainly due to the introduction of the monoclonal anti-CD20-antibody rituximab combined with the standard CHOP-regimen. However, very old DLBCL patients (above the age of 80 years in each time period, corresponding to 20.5 % and 23 %, respectively, of the entire DLBCL population diagnosed during these two periods. Performance status ≥ 2 was 48 and 47 % in each period and no difference in aIFI was found. Fifty-three % in the post-R period were treated with a curative intent compared to 37 % in the pre-R period (p<0.05). The estimated 3-year PFS was 42 % in the post-R period and 20 % in the pre-R period (p=0.045). Similarly, the estimated 3-year OS in the post-R group was 40 % and 19 % in the pre-R group (p=0.01). Conclusions. After the introduction of R-CHOP, both PFS and OS was improved for a population of very elderly DLBCL patients. Based on our data, high age by itself should not be a reason to exclude the patient from an effective treatment with immunochemotherapy.

**0940**

**CONCURRENT CHROMOSOMAL BCL2 AND MYC TRANSLOCATIONS IN LARGE B CELL LYMPHOMA**

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**Background.** Concurrent chromosomal BCL2 and MYC translocations involving the BCL2 and MYC protooncogenes in Large B Cell Lymphoma (NHL) (double-hit, DH) recently, have received increased attention (2008 WHO classification, B cell lymphoma unclassifiable with features intermediate between DLBCL and BL). Patients with DH lymphomas express high levels of a number of pro-survival and anti-apoptotic parameters, including elevated LDH, bone marrow and CNS involvement, and a high IPI score. All studies on larger series of patients suggest a poor prognosis, also if treated with RCHOP or high-intensity treatment modalities. Aims. We conducted a retrospective study of DLBCL to evaluate the frequency of double-hit B translocations in DLBCL and to analyse pathologic and/or clinical features correlated with the presence of a double-hit translocations. Methods. DLBCL samples, diagnosed according to the WHO criteria of 2008 and derived from 93 patients treated with R-CHOP or HD, have been subjected FISH using commercial break-apart probes for BCL2 and MYC. Clinical data were collected from patient files. Results. Double-hit BCL2/MYC translocations were detected in 9 of 93 cases (10%); 7 DLBCL, 1 BCLU, 1 unclassified. All double-hit DLBCL were GCB immunophenotype and showed varying morphology. Ki-67 index ranged from 15-95%. Characteristic clinical parameters included a high IPI score, a high stage an extranodal involvement with CNS involvement(4/9). Furthermore DH was also associated with an inferior OS. Conclusions. Our results suggest that DH is more frequent than previously estimated but could not be identified only by morphology or proliferation rate.

**0941**

**HISTORY OF MYELOID MALIGNANCIES IN RELATIVES OF PATIENTS WITH WALDENSTRÖM MACROGLOBULINEMIA AND IG-MONOCYCLONAL GAMMAPATHIES**

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**Background.** The etiology of Waldenstrom macroglobulinemia (WM) is unknown. A possible role for genetic factors has been suggested by reports of familial clustering of WM. Familial cases reported so far show two patterns of aggregation: multiple cases of WM only, or mixed B-cell disorders. Aim. The aim of this study was to evaluate the incidence and characteristics of hematologic disorders among family members of 212 unrelated patients with IgM-monoclonal gammapathies diagnosed and followed at our Institution from 1986 to 2010. Methods. An interview-based investigation of family history was performed to identify a history of hematologic diseases among relatives. Diagnosis of WM, IgM-monoclonal gammapathies of undetermined significance (MGUS), and IgM-related disorders (IgM-RD) was made according to the Consensus Panel Recommendations from the Second International Workshop on WM (Owen et al., 2003) for patients diagnosed from 2003 onward. The same criteria were retrospectively applied to patients diagnosed before. Results. We analyzed 212 patients with IgM-monoclonal gammapathies classified as WM, MGUS/IgM-RD (146 patients). The median age was 63 years (range: 19-92), 125 were males and 87 females. A familial history of hematologic disorders in one or more relatives was reported by 31 patients (15%), totaling 40 affected relatives. Hematologic disorders were diagnosed in 28 first-degree and 16 second- or third-degree relatives. The median number of relatives affected per family was 1 (range: 1-5). Patients with familial IgM-monoclonal gammapathies were significantly younger as compared to those with sporadic disease (53 versus 64 years, p<0.0001). The proportion of familial cases was higher among patients with WM as compared to those with MGUS/IgM-RD (25% versus 11%, p=0.02). Specific information regarding the type of hematologic disease in the family members was not available in one case. Among the remaining 39 cases, the diagnoses were as follows: WM (=4), MGUS (=9), non-Hodgkin’s lymphoma (=8), chronic lymphocytic leukaemia (=3), Hodgkin’s lymphoma (=1), hairy cell leukaemia (=1), multiple myeloma (=1), acute lymphoblastic leukaemia (=1), acute myeloid leukaemia (=1), chronic myeloid leukaemia (=1), polycythemia vera (=2), essential thrombocytopenia (=1). According to the previously reported patterns of aggregation, we observed multiple cases of WM only in 3 families (10%) and mixed B-cell disorders in 18 (60%). In the remaining 9 families (50%) we found clustering of IgM-monoclonal gammapathies with hematologic myeloid malignancies. Conclusions. In this study we found that 15% of patients with IgM-monoclonal gammapathies or IgM-MGUS/IgM-RD have a familial history of hematologic disorders. Familial cases are more common among WM patients than in other IgM-monoclonal gammapathies and are characterized by younger age at diagnosis. In addition to the two patterns of aggregation described so far, we identified a subset of IgM-monoclonal gammapathy patients with a familial history of myeloid malignancies. This suggests a thorough investigation of family history in patients with IgM-monoclonal gammapathies, encompassing also myeloid malignancies. Further investigations might clarify whether familial clustering reflects a genetic predisposition rather than exposition of family members to the same environmental risk factors.
volvedment without any other disease localization. PBMMZL harbour many similarities with SMZL besides splenomegaly. Aim To compare SMZL and PBMMZL on the basis of clinical and laboratory characteristics, morphology and immunophenotypic data. Patients and Methods. 23 SMZL and 21 PBMMZL patients were included in the present study. Clinical and laboratory features were recorded. Blood and bone marrow mononuclear cells were studied by flow cytometry for the mAbs CD5, CD23, CD10, CD25, CD38, CD11c on CD19+ cells, while bone marrow (BM) biopsies were evaluated using morphologic and immunohistochemical methods. Results. Clinical and laboratory features were very similar between the two groups (table 1), except that cytopenias and elevated LDH were more common in SMZL patients. Immunophenotypic features of blood and bone marrow mononuclear cells were also similar. The percentage of BM infiltration was 40 (15-85) and 25 for SMZL and PBMMZL respectively. The histological pattern of bone marrow infiltration was variable, with the nodular infiltrates being the most frequent and typical pattern in both groups (78% for the SMZL and 62% for PBMMZL). Intrasinusoidal infiltration was observed in both groups usually in association with other patterns of infiltrates (48% for SMZL and 38% for PBMMZL). Cytological aspects were very heterogeneous in both groups with several types usually associated in varying proportions: small lymphocytes, centrocyte-like cells, small cells with plasmacytoid differentiation, monocyteid cells and variable content of medium to large cells. Well-formed germinal centres were identified in 48% of SMZL cases in contrast to only 1 case with PBMMZL. In all the cases the immunophenotypic profile of BM neoplastic population (CD20+ strong, CD79a+, CD3−, CD5−, CD10−, cyclin-D1−, BCL-6−) was consistent with a marginal zone-derived neoplasm. PBMMZL patients presented a very indolent clinical course. Only 3 patients have required so far therapy while all SMZL patients were in need of therapy at diagnosis. No death was recorded in the PBMMZL group at a median follow up time of 36 months (12-105), while only 3 SMZL patients have died (3 disease related) at a median follow up time of 72 months (16-181). Conclusions. PBMMZL presents many similarities with SMZL, although the former presents a more indolent clinical course and is not complicated by splenomegaly.

Table 1. Pts characteristics with SMZL and PBMMZL

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SMZL (%)</th>
<th>PBMMZL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>64 (37-77)</td>
<td>64 (48-78)</td>
</tr>
<tr>
<td>B symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td></td>
<td></td>
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<tr>
<td>Neutropenia</td>
<td></td>
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<tr>
<td>Lymphopenia</td>
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0943 NEW MODIFIED PROPHYLACTIC SCHEME AGAINST LIPOSOMAL CYTARABINE (DEPOCYTE®)-INDUCED ARACNOIDITIS IN ADULT LYMPHOMA PATIENTS

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Background. Liposomal cytarabine (DepoCyte®) is a slow-release formulation of cytarabine designed for intrathecal administration, ensuring prolonged drug exposure. In all clinical studies, chemical arachnoiditis, a syndrome manifested primarily by nausea, vomiting, headache and fever, was a common adverse event. To reduce the incidence and severity of chemical arachnoiditis, all patients must be treated with demethylsone 4 mg bid orally or intravenously on days 1 to 5 of each cycle beginning concurrently with administration of DepoCyte. Aim We designed a new prophylactic scheme against Liposomal Cytarabine-induced arachnoiditis in patients with lymphoma to improve adherence to concomitant oral demethylsone. Patients and Methods. Patients were consecutively recruited from 7 Spanish institutions. Thirty-three adult patients with lymphoma, median age of 57 years (27-79). Type of lymphoma: diffuse large B-cell lymphoma 24 pts, mantle cell lymphoma 3 pts, Burkitt 2 pts and other types 4 pts. New prophylactic scheme consisted of oral dexamethasone 4 mg by lumbar puncture immediately after the injection of DepoCyte and demethylsone 4 mg bid orally on days 1 and 2. Toxicity was graded according to Common Terminology Criteria for Adverse Events, version 3.0. Results. Reasons for Liposomal Cytarabine administration were: therapeutic in 12 pts (36%) and prophylactic in 21 (64%). A total number of 78 injections of DepoCyte were administered. The median number of doses per patient was 2 (range, 1-6). Liposomal cytarabine was generally well tolerated. Overall, 15 pts had some type of adverse event (all but one in prophylaxis and 9 of them with the first injection). No toxicity was observed in 56 administrations. In 22 administrations (28%) some type of toxicity was recorded for a total of 54 adverse events. Most of these events were transient and resolved spontaneously. Adverse events are listed in the Table. Only 2 events were considered clinically relevant: 1 patient with headache grade 3 with diplopia grade 2 and other patient with arachnoiditis grade 2. Interestingly, the patient who had arachnoiditis fully recovered and received 3 additional administrations of DepoCyte with a dose reduction of 25 mg. He was given the same prophylactic scheme against DepoCyte-induced arachnoiditis showing excellent tolerance and without any relevant symptoms. Regarding efficacy, all evaluated patients cleared cerebrospinal fluid and/or improved clinical symptoms. At last follow-up, nine patients have died, being the main causes of death: 5 systemic lymphoma progression, 1 systemic plus neurologic progression, 1 isolated neurologic progression, 1 concomitant solid tumour and 1 neutropenic sepsis. Conclusion. Administration of concomitant intrathecal demethylsone and oral demethylsone only for two days appears to be safe and well tolerated prophylactic scheme against Liposomal Cytarabine (DepoCyte®)-induced arachnoiditis in adult patients with lymphoma. This strategy reduces the number of days with oral demethylsone, while also might improve adherence allowing to administer the scheduled number of DepoCyte injections.

0944 SPLENIC MARGINAL ZONE LYMPHOMA: CHARACTERISTICS AND TREATMENT


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Splenical marginal zone lymphoma (SMZL) characterized with primary spleen involvement and indolent course. SMZL is rare and found in less than 1% of all Non-Hodgkin’s lymphomas. The aim of our study was to
find stricter definition for SMZL and to determine the most effective treatment strategy. Aims: Materials and Methods. Eighty-six patients (55 males (58%) and 53 females (62%)) between January 2001 and February 2011 were included in the study. The median age was 59.6 years (ranging from 16 to 86 years). Splenomegaly was noted in all cases. Visceral lymphadenopathy was detected in 44 (50%) patients and was shown as an increase of liver size and splenic hilar lymph nodes. In the laboratory data analysis, 51 patients (56%) had thrombocytopenia, 65 (76.7%), leukopenia - in 30 (35%), leukocytosis - in 39 (45.3%). Absolute lymphocytosis was detected in 2/8 of patients. High LDH was observed in 46 patients (53.5%). M-component detected in 35 patients (40.7%). Viral hepatitis markers were positive in 12 cases (14%). Aberrant expression of chromosome 3, 7, 12, 13, 15, 18 and in 3 cases (654.12) was sequence revealed. In order to determine the role of surgical treatment in patients with SMZL, we compared two groups of patients selected on the basis of the first-line therapy. The first group included 17 patients who completed chemotherapy in the first line, and the second group consisted of 47 patients in whom splenectomy was first-line treatment. Results and Discussion. All patients who received chemotherapy as a first-line treatment demonstrated little clinical and hematologic effects, but sooner or later recurrent free period than those in the second group. On average, progression of disease occurred during 6-8 months in all patients and manifested with spleen size increase and progression of cytopenia. One patient from the first group died due to infectious complications. Splenectomy was performed to all patients in this group with good clinical results obtained. In patients who underwent splenectomy as first-line therapy, we obtained a good clinical response. In the second group 1 patient died from disease progression. Overall survival rate in both groups about 98% with 9 years follow-up. However, we obtained significant differences in the progression-free survival: in the first group (chemotherapy in first line) relapse rate during 9 years was about 0%, meanwhile the second group (splenectomy in the first line) it was 50% (p = 0.01). Conclusion. SMZL heterogeneous disease and characterized by different clinical manifestations, chromosomal abnormalities, secretion of M-component, often aberrant immunophenotype, but in all cases, splenomegaly played a leading role in the treatment strategy. Effectiveness of chemotherapy was noted in the first-line therapy compared with chemotherapy. It was demonstrated with statistical significance that splenectomy as a first-line therapy in these patients was three times more clinically effective than chemotherapy subsequent to surgery.

**0945**

**ADDITION OF RITUXIMAB TO REDUCED DOSE CHOP CHEMOTHERAPY IS FEASIBLE IN ELDERLY PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA**

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**Background.** Recently, rituximab plus dose intensified CHOP chemotherapy regimens have been shown to be effective in elderly patients with diffuse large B cell lymphoma (DLBL). However, hematological toxicities and treatment related mortality were hard to be neglected in terms of relatively poor performance in elderly patients. Rituximab is effective immunomodulatory drug in DLBL, if that, addition of rituximab to reduced dose CHOP chemotherapy may be appropriate for elderly patients with DLBL. **Aims.** To investigate outcomes and toxicity profiles of addition of rituximab to reduced dose CHOP chemotherapy in elderly patients with DLBL. **Methods.** Patients aged 60 years and over have been enrolled consecutively between January 2005 and December 2009. **Results.** Reduced dose CHOP chemotherapy consisted cytosphosphamide (600 mg/m² intravenously), doxorubicin (30 mg/m² intravenously), and vincristine (1 mg intravenously) on day 1, and prednisone (40 mg orally) given on day 1 to 5. Patients were treated every 3 weeks for 6 to 8 cycles. Rituximab was administered at a dose of 375 mg/m² on day 1 of each cycle. After a median follow-up of 37 cases were included in this study. Median age was 69 years old (range, 61-85 years old) and males were 44 (52.4%). 65 patients received at least 6 cycles of chemotherapy and 19 patients dropped out from these regimens early because of treatment-related death (4 patients), loss of follow up (4 patients), progression of disease (4 patients), or refusal of further treatment (7 patients). Mean cycles of modified R-CHOP chemotherapy was 5.96 (range, 1-8). Overall response rate was 89.3% (CR rate, 66.7%; PR rate, 22.8%). Estimated 5-year event-free survival rate was 61.5% ± 6.2% and overall survival rate was 78.3% ± 5.2%. Main hematological adverse events were 21.4% in grade 3/4 neutropenia and 3.6% in grade 3/4 anemia. Among non-hematological toxicities, grade 3/4 asthenia was reported 3.6% and other toxicities were tolerable. **Conclusions.** Addition of rituximab to reduced dose CHOP chemotherapy is an effective and tolerable treatment regimen in elderly patients with DLBL.
Non-Hodgkin lymphoma - Clinical 3

0947
SWITCHING BCL6 POSITIVE TO NEGATIVITY IN RELAPSING DIFFUSE LARGE B-CELL LYMPHOMA
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Background. Diffuse large B-cell lymphoma (DLBCL) is the most complex and heterogeneous lymphoma in adulthood presented as a biologically and clinically distinct subtypes including germinal centre B-cell-like (GC-B) and activated B-cell-like (ABC). In DLBCL BCL-6 is associated with the germinal centre subtype and has a good response to modern chemotherapy. It is known that BCL-6 expression is a favorable prognostic factor in DLBCL. However, the effect of BCL-6 expression on relapse of the DLBCL was not studied. Aim. To show BCL-6 gene expression changes and biological response in relapsed DLBCL and their patterns which include: LDH, B2M, CRP, Fe, ferritin, TIBC, hemooglobin, uric acid and International Prognostic Index (IPI). Methods. We investigated 45 (21F/20M) patients with relapsed DLBCL and role of immunohistochemically determined Bcl-6 tested in 22 patients. All patients were treated with R-CHOP protocol. We also investigated biological parameters such as LDH, B2M, CRP, Fe, ferritin, TIBC, hemooglobin, uric acid as well as IPI at the presentation of DLBCL and at the time of relapse. Results. Mean age of female patients was 53.1±12.7 vs. male 52.8±12.1 and overall age of patients was 53.0±12.3SD years with no significant difference. Investigated relapsed group of 41 patients with DLBCL had own control at the beginning of diseases. From 22 immunohistochemically (IHC) investigated patients for Bcl-6 protein expression, 19/22 was positive and 3/22 was negative at the beginning. DLBCL was diagnosed in gastrointestinal tract (13), lungs (5), liver and spleen (7) lungs (5), CNS (4), ovarian (2), vertebral (1), and other sites (6). Expression of the single gene Bcl-6 strongly predicts poor outcome of relapsed DLBCL. In human genome it is very rare that single gene can predict outcome of diseases such as Bcl-6, but also can predicts relapse as showed in this report. Switching positive into negative Bcl6 expression was strong indicator of DLBCL relapse.

0948
OUTCOME OF PATIENTS WITH DLBCL AFTER ADDITION OF RITUXIMAB TO CHOP: A POPULATION-BASED TIME PERIOD ANALYSIS IN MANITOBA
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TO CHOP: A POPULATION-BASED TIME PERIOD ANALYSIS IN MANITOBA
OUTCOME OF PATIENTS WITH DLBCL AFTER ADDITION OF RITUXIMAB

Summary/Conclusion.

0949
IMPACT OF AGE GROUP ON FEBRILE NEUTROPENIA (FN) RISK ASSESSMENT AND MANAGEMENT IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH R-CHOP REGIMENS
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Background. Primary prophylaxis with granulocyte-colony stimulating factor (G-CSF) is recommended by the ASCO and EORTC guidelines to support chemotherapy delivery in patients with a high (≥20%) risk of FN. R-CHOP regimens used to treat aggressive non-Hodgkin lymphoma (NHL) are associated with high FN risk, and G-CSF prophylaxis is particularly important in dose-dense R-CHOP-14 protocols to support full chemotherapy dose intensity. Older age is a known additional risk factor for FN. IMPACT NHL was an observational study designed to evaluate current clinical practice with respect to FN risk assessment in NHL patients treated with R-CHOP. Aims. To evaluate the impact of age group on FN risk assessment, G-CSF use, FN incidence, and chemotherapy delivery in patients with DLBCL receiving R-CHOP regimens. Methods. The study was conducted in Europe and Australia. All patients gave informed consent where required. Physicians assessed each patient’s FN risk based on the planned chemotherapy regimen and individual patient risk factors. The primary outcome measure was the proportion of patients who did not receive at least one cycle of CHOP or R-CHOP over a period of 36 months pre and post funding of Rituximab for this indication. Funding of Rituximab was approved for patients age 60-80 on 28 October 2002 and for all age groups on 22 June 2004. The proportional odds model was used to test for independent effect of treatment in the post rituximab period on OS, after controlling for age and sex. Data was analyzed using SAS version 9.1. Results. Patient characteristics: Time-Period Analysis- Total number 257 (male 118); Pre-rituximab 112 (median age 62 years, median follow up 45 months); Post-rituximab 125 (median age 65 years, median follow up 45 months). Drug-Period Analysis- Total number 196 (male 97); Pre-rituximab 94 (median age 62 years, median follow up 56 months); Post-rituximab 102 (median age 64 years, median follow up 45 months). After controlling for age and gender, there was significant improvement in OS in the Post-rituximab era for both the Time-Period Analysis (hazard ratio, 0.53; 95% CI 0.31 to 0.92; p = 0.02) and the Drug-Period Analysis (hazard ratio, 0.52; 95% CI 0.28 to 0.98; p = 0.04). Conclusion. The study shows a significant improvement in OS in patients with DLBCL treated in multiple Community Cancer Centers and two Academic Centers in the Province of Manitoba, after the addition of Rituximab to CHOP as primary therapy.
Table 1. Outcomes by chemotherapy regimen and age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number of patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;65</td>
<td>120</td>
<td>60%</td>
</tr>
<tr>
<td>≥65</td>
<td>162</td>
<td>40%</td>
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Results. After recruitment of 171 pts the planned final analysis was performed on an intention to treat basis. Complete data sets of 165 pts were evaluable. At study entry, 120 pts (74%) were in CR, 2 pts (2%) in unconfirmed CR and 41 pts (25%) in PR. Histological subtypes included diffuse large cell lymphoma (67 pts), follicular lymphoma (35 pts), mantle cell lymphoma (18 pts), small lymphocytic lymphoma (16 pts), marginal zone lymphoma (7 pts), Burkitt’s lymphoma (5 pts), and other lymphomas (15 pts). After a median follow up of 28 months, EFS (HR 0.50, 95% CI 0.25-1.05, p=0.047) and RFS (HR 2.52, 95% CI 1.15-5.70, p=0.03) were superior for the maintenance group. In regards to diagnostic subgroups, EFS was in particular prolonged in pts with mantle cell lymphoma (p=0.055, one sided logrank test) and to a lesser extent in pts with follicular lymphoma (p=0.16) and diffuse large cell B cell lymphoma (p=0.18). Relapse occurred more often in the observation group than in the treatment group, however this effect was not significant (relapse rate observation group/treatment group = 2.31, 95% CI 0.66-7.5, p=0.08). There was no difference in OS between the two groups (p=0.74).

1. Introduction.

ORAL CYCLOPHOSPHAMIDE AND RITUXIMAB IN THE TREATMENT OF MARGINAL ZONE LYMPHOMA

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Introduction. MZL accounts between 5% and 17% of all non-Hodgkin lymphomas. MZL are most often very indolent malignancies that usually present with limited stage disease, which may be controlled with local treatment, with 10 years survival approximately 75-80%. Standard treatment option for stage I-II disease has not been yet established because the paucity of prospective trials, the heterogeneity of treatment and short follow-up and the indolent nature of disease justify a conservative approach. Patients with systemic stage III-IV disease should be considered for a more aggressive treatment. However a standard chemotherapeutic approach for MZL is missing. Aim. We are reporting a retrospective study on the use of oral cyclophosphamide and rituximab in the treatment of advanced stage MZL patients. Cyclophosphamide is a nitrogen mustard alkylating agent identified as a lymphocyte cytotoxic agent. It determines DNA crossing between (interstrand crosslinkages) and within (intrastrand crosslinkages) DNA strands at guanine N7 positions in irreversible manner, leading to cell death. Adding rituximab allows targeting CD20 MZL positive cell, obtaining a synergistic effect on lymphoma cells. Patients and Results. 38 patients with MZL included in the study and received 100 mg/die orally CTX for 15 days a month and 375 mg/sqm rituximab on day 8 of CTX therapy, monthly, for a 6 months treatment program. 31 patients are retrospectively valuable. The global overall response rate was 90.5%, with 51.6% complete response (CR) and 38.7% partial response (PR). The remaining 9.7% patients obtained a stable disease or a disease progression at the end of treatment. The principal side effects were recorded with rituximab use, with 4 patients that presented infusional reactions despite paracetamol and antihistamine premedication. One patient experienced herpes zoster reactivation. Patients achieving a response (CR or PR) underwent to follow-up, with a median of 10 months (mean 15). PFS presented a median of 23 months, and no statistically significant differences documented between patients achieving a CR or a PR, due to short follow-up. All patients underwent at diagnosis to bone marrow aspiration and further analysis for IgVH rearrangement by PCR, to monitor minimal residual disease after therapy. Of the 31 pretreatment evaluable patients, 20 were positive, 11% negative at PCR. After treatment 10 patients were positive, 15 negative and 8 not evaluable. All PCR negative patients remained negative at the end of therapy. With regard to FFS, in the PCR negative subgroup we recorded 18.7% disease relapse, while 57.1% in the positive subgroup, confirming a predictive role of molecular biology status at the end of treatment (see figure). Conclusions. In the absence of comparative trial, it is difficult to know if any particular regimen should be preferred for the treatment of MZL. Oral cyclophosphamide combined

Survival (OS). EFS and OS were analysed using an asymptotic log rank test. RFS using a competing risk R using Fisher’s exact test.
with rituximab immunotherapy is effective, safe and reliable in the treatment of MZL. Minimal residual disease condition at the end of treatment may have a predictive role of disease relapse. Prospective randomized studies are necessary to validate our results and to define a standard approach in advanced stage MZL patients.

0952 CLINICAL IMPACT OF EBV-ENCODED LMP1 IN EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE

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Background. Extranodal NK/T-cell lymphoma, nasal type (ENKL) is a rare disease and more frequently develops in East Asia than in Western countries. Although more than 95% of the cases harbor Epstein-Barr virus (EBV) genome in the neoplastic cells, little is known regarding the roles of EBV-associated gene products in ENKL. Aims. To elucidate detailed expression profiles of EBV-associated gene products and their impact on clinical behavior in ENKL. Methods. ENKL cases referred to Juntendo University Hospital between August 1996 and June 2010 were retrospectively studied. All cases were diagnosed according to the latest World Health Organization classification. Expressions of EBV-associated gene products were evaluated by immunohistochemistry in formalin-fixed, paraffin-embedded tissue sections. This study was conducted with approval of the Institutional Review Board of Juntendo University, and informed consent was obtained in accordance with the Declaration of Helsinki. Results. Total 30 ENKL cases were analyzed. The cases consisted of 19 males and 11 females, and the median age at diagnosis was 62 years (range 27-85 years). All cases were positive for EBER1 and lacked EBNAA2 expression. ZEBRA was detected in four cases (13.3%), LMP1 and LMP2A were detected in 22 cases (73.3%) and 12 cases (40.0%) respectively. However, co-expression of LMP1 and LMP2A was observed in 10 cases (33.3%). LMP1-positive cases showed more limited disease and had a survival advantage in ENKL.

0953 RELAPSE PATTERN AND PROGNOSTIC FACTORS OF PRIMARY CNS LYMPHOMA

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Background. Primary central nervous system lymphoma (PCNSL) rarely relapses in extracranial sites, but there has not been a specialized guideline for follow-up evaluation. High-dose methotrexate based chemotherapy and high-dose chemotherapy (HDC) followed by autologous stem cell transplantation (ASCT) were shown as alternative strategies for PCNSL recently, but data on prognostic factors with these treatment scheme are limited. Aims. The aim of this study was to evaluate the pattern of relapse and investigate prognostic factors for PCNSL with single institution experience. Methods. Between November 1995 and August 2010, 67 patients with newly diagnosed PCNSL at the Asan Medical Center, Seoul, Korea were included. Results. The median age was 54.5 years (range, 26-77 years) and 55 (78.6%) patients had intracranial lesions only. Nine patients had leptomeningeal involvement, 2 had ocular lesions, and one had spinal cord lesion. Twenty-nine patients (43.3%) were treated with chemotherapy only, 13 (19.4%) with chemotherapy followed by whole brain radiotherapy (WBRT), while 20 (29.8%) were given HDC followed by ASCT. Two patients received palliative WBRT only and 3 received best supportive care. While all patients achieved CR (complete response, 76.2%) or PR (partial response, 23.8%) with ASCT, overall CR rate of chemotherapy only and chemotherapy followed by WBRT were 64.8% and 84.6%, respectively. No systemic relapse was noted among 27 patients experiencing relapse; intracranial lesion only in 23 patients, 3 with leptomeninginal involvement and one with ocular relapse. Median overall survival (OS) and failure free survival (FFS) of all patients were 35.8 and 13.1 months, respectively. Age< 60 years (44.9 ± 12.41 versus 27.0 ± 6.93 months, p=0.040) and Eastern Cooperative Oncology Group performance status of patients that received ASCT had longer OS (58.6 ± 21.48 versus 33.3 ± 8.65 months) but without statistical significance (p=0.083), while they had significantly better FFS (35.5 ± 19.83 versus 9.9 ± 2.96 months, p=0.013). Conclusions. Considering no systemic involvement of relapsed PCNSL in current study, evaluation with computed tomography or positron emission tomography to investigate extracranial sites might not be necessary in PCNSL patients. Age and FFS still retain prognostic significance irrespective of treatment scheme, and ASCT could lead to improve response rate and FFS.

0954 TOXICITY ADAPTED HIGH-DOSE THERAPY IN MANTLE CELL LYMPHOMA PATIENTS, USING SCHEMES EPOCH, HMA AND GIDIOX, AUTOLOGOUS STEM CELL TRANSPLANTATION AND RITUXIMAB

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Background. Mantle cell lymphoma (MCL) is aggressive B-cell neoplasm that progresses predominantly among elderly persons. HDC-like schemes are effective in remission induction, but progression free survival is short with median overall survival of 3-5 years. Upfront use of high-dose cytarabine (12 g/m2), autologous and rituximab at all stages of therapy is the most effective treatment but possible only with younger patients. Decrease in AraC doses to 4 g/m2 per cycle significantly reduced toxicity. Prominent efficacy of gemcitabine-oxaliplatin combinations and irinitecan in relapsed and refractory MCL patients allowed including these drugs in first-line treatment in cases when the scheme R-HD-Meta-AraC (Romanguera J. 2005) is impracticable. Aim. toxicity and efficacy assessment of schemes R-EPOCH/R-GIDIOX and R-EPOCH/R-HMA in primary MCL patients eligible for autologous stem cell transplantation. Methods. Since May 2008 17 untreated MCL patients (average age 65 years, 12 males and 5 females, IMPI: 35% high, 30 % intermediate, 35% low risk) were enrolled. After first R-EPOCH cycle (Wilson W. 2003) patients were stratified according to toxicity they had received either R-EPOCH/R-HMA or R-EPOCH/R-GIDIOX (gemcitabine 800 mg/m2 days 1 and 4, oxaliplatin 120 mg/m2 days 1 and 2, irinotecan 100 mg/m2 days 3, dexamethasone 10 mg/m2 days 1-5, ifosfamide 1000 mg/m2 days 1-5) for those who got higher hematological toxicity (grade 4 for more than 3 days). Depending on the terms of response, patients received 6-8 cycles of immunotherapy and autologous stem cell transplantation (ASCT) in responders or purging by rituximab before harvest, and HDC followed by autologous stem cell transplantation. Patients with residual tumor after autologous stem cell transplantation were consolidated with local radiotherapy. MKD was diagnosed and controlled by flow cytometry. Rituximab maintenance was performed every three months for 2 years. Results. 29 GIDIOX cycles were analyzed with evidence that GIDIOX is less intensive and toxic then HMA, but in our selection hematologic toxicity in R-GIDIOX and R-HMA arms were similar due to less fit patients in R-GIDIOX arm, except thrombocytopenia, that was more prominent and lasting in R-HMA arm. Main non-hematologic toxicity of GIDIOX was hepatic, with ele-
vated aminotransferases grades 1-2 and 3 in 48% and 3% of cycles respectively, without clinical signs. Complete responses were obtained for 16 out of 17 patients after a median of 6.5 cycles in both arms. The sources of stem cell were PB in 15 patients and BM in one case of harvest failure after GIDIOX. Median number of collected stem cells was 4.07×10^6/kg. 8 patients achieved CR (MRD-) among 9 ones in HMA arm, 1 induction death after first HMA cycle (acute renal failure and septic shock), and one relapse in 8 months after autoSCT, with observation from 1 to 15 months. GIDIOX arm included 8 patients: OR 100%, 7 CR (including 1 patient who achieved CR after radiotherapy), one patient with partial remission continues to get treatment, observation from 1 to 23 months, without relapses. Conclusion. GIDIOX scheme is less toxic than HMA and equally effective in response induction and mobilizing, so it could be recommended for those in whom high-dose Ara-C and methotrexate can potentially cause severe adverse consequences.

0955 FEASIBILITY OF THE TNM-BASED STAGING SYSTEM OF OCULAR ADNEAL EXTRANODAL MARGINAL ZONE LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT LYMPHOMA)

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Background. Malignant lymphomas of the ocular adnexa account for 1-2% of non-Hodgkin lymphomas and 8% of extranodal lymphomas. Although immunohistochemical and molecular analyses have resulted in ocular adnexal lymphoma (OAL) being diagnosed more frequently, previous studies do suggest that there has been a true and inexplicable rise in the incidence of these tumors in the last decades. Among OALs, the most common subtype by World Health Organization (WHO) classification is marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Several studies have shown that the relative incidence of ocular adnexal MALT lymphoma (OAML) varies among different populations. The proportion of OAML among primary OALs is higher in Korea (86-96%) than in Western countries (50-78%). Although the prognosis of patients with OAML is generally favorable, some situations such as higher clinical stage, nonconventional primary tumor involvement, and bilaterality at presentation, and/or have been associated with a worse prognosis. However, based on the Ann Arbor staging system, two-thirds of OAMLs have been recorded as stage IE tumors, because the Ann Arbor staging system does not distinguish among OAMLs based on anatomic location, extent of primary tumor infiltration, multicentricity, or bilaterality. The Ann Arbor staging system is not particularly useful in determining the prognosis for these patients. The American Joint Committee on Cancer (AJCC) proposed the tumor, node, metastasis (TNM)-based clinical staging system for primary OAL to overcome the weak points of the Ann Arbor staging system, but the clinical impact of TNM-based staging on primary OAML has not been determined. This study is novel and demonstrates the feasibility of the TNM-based staging system for OAML. Methods. We performed this study to evaluate the feasibility of the TNM staging system for ocular adnexal extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (OAML). The data form 66 total eyes from 54 patients with biopsy-confirmed OAML according to World Health Organization classification were retrospectively analyzed. Results. Using the TNM staging system, we reclassified all patients into two categories: (1) T1N0M0 stage group (n=26), for patients with lymphoma involving only the conjunctiva; and (2) above T1N0M0 or bT1N0M0 stage group (n=29), for patients with lymphoma extending to the orbit, eyelid, or adjacent structures, and/or bilateral OAML. After a 24-month median follow-up period for all patients, the T1N0M0 group revealed higher progression-free survival (PFS) than the above T1N0M0 or the bT1N0M0 group (P=0.041). In a separate analysis of only 50 patients categorized as Ann Arbor stage IE, the T1N0M0 group demonstrated higher PFS (100%) than the above T1N0M0 or the bT1N0M0 group (84.7%; P=0.067). Conclusions. Our data show that the poor prognostic group classified as Ann Arbor stage IE can be further distinguished by using the TNM staging system. Thus, further studies to develop treatment strategies for reducing relapse after treatment for OAML should use the TNM staging system.

0956 TREATMENT OF BURKITT LYMPHOMA IN ADULTS USING AN ADAPTED PEDIATRIC ALL PROTOCOL: A SINGLE CENTER EXPERIENCE

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LUMC, Leiden, Netherlands

Background. Burkitt lymphoma is an aggressive B-cell malignancy that accounts for 1% to 2% of all adult lymphomas in Europe. Treatment consists of dose-intensive, multi-agent chemotherapy and is highly effective but associated with considerable toxicity and treatment-related mortality. Several therapeutic strategies are currently in use, but the optimum treatment has yet to be defined. Aims. We report the results of a series of adult patients treated for Burkitt lymphoma with a protocol adapted from the LMB regimens of the Société Française d’Oncologie Pédiatrique. Methods. Twenty-three patients, 18 male and 5 female, were diagnosed with Burkitt lymphoma between 1997 and 2009. Median age was 39 years (14 to 74 years). No patients were HIV-positive. Fifteen patients presented with stage III-IV disease, 8 of which had central nervous system involvement. According to the revised WHO 2008 criteria 5 cases would now be classified as B-cell lymphoma unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. All patients were treated with an adapted LMB protocol consisting of an initial cytoreductive phase (COP), followed by 2 induction cycles (COPADM), 2 consolidation cycles (CYVE), and 4 maintenance cycles. Fourteen patients also received rituximab. Median follow-up was 31 months. Results. The adapted LMB protocol was well tolerated by most patients. Frequent toxicities included myelosuppression, neutropenic fever, and grade I-II mucositis and vomiting. Three patients experienced grade III mucositis, 1 patient grade III neuropathy, and 2 patients nephrotoxicity (1x grade II, 1x grade III). Tumor lysis syndrome occurred in 3 patients. Eighteen out of 25 patients achieved complete response (CR). Four patients had refractory disease. One patient achieved a partial response and received an autologous stem cell transplantation (SCT). He relapsed several years later and died during salvage therapy. Three patients underwent myeloablative allogeneic SCT: 2 in 1st CR because of extensive disease and the presence of a sibling donor, 1 following relapse after initial CR. Two of these patients died from treatment-related complications. The remaining 16 patients are alive and in CR as of today, including 4 out of 5 patients with B-cell lymphoma unclassifiable. The 2-year event-free survival and overall survival rates were 73.9% and 78.3% respectively. 2-year overall survival rates were significantly better for patients younger than 40 years (91.7% versus 50.0%), patients with early disease (100% versus 60.0%), and patients with low or low-intermediate IPI scores (100% versus 50.0%). Conclusions. The adapted LMB protocol is an effective regimen in adult patients with Burkitt lymphoma, Burkitt leukemia, and B cell lymphoma unclassifiable, with a Burkitt-like phenotype. Survival rates are similar to the outcomes in case series using other treatment regimens such as CODOX-M/IVAC. However, compared to these studies we report a relatively low incidence of grade III-IV mucositis and grade III-IV neuropathy. The role of allogeneic SCT for Burkitt lymphoma should remain limited to salvage therapy.

0957 MSCT FOLLOW UP IN PATIENTS WITH MALIGNANT LYMPHOMA: DOES SEMI-AUTOMATED VOLUMETRY IMPROVE THERAPY RESPONSE CLASSIFICATION COMPARED TO MANUAL MEASUREMENTS?

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Background. Standardized staging and response assessment are crucial in lymphoma clinical trials in order to afford assessment of treatment strategies and facilitate comparison among studies. Aims. Impact of semi-automated volumetry compared to unidimensional measurements on therapy response classification in CT follow-up of malignant lymphoma. Methods. MSCT scans of 65 patients with malignant lymphoma prior to therapy (baseline) and after 2 cycles of chemotherapy.
OUTCOME OF NON-HODGKIN LYMPHOMA (NHL) AND CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) IN THE SPANISH BENDAMUSTINE REGISTRY

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Background: Bendamustine (B) is a purine analog/alkylator hybrid with antitumor activity. B is currently licensed by EMEA for use in follicular NHL. Adult sporadic BLL is a rare and highly aggressive malignant. It is known to strongly express surface CD20 antigen. Recent data indicates that the outcome of adult BLL patients can be improved by the addition of anti-CD20 antibody rituximab to multi-agent chemotherapy. The population based outcome data of BLL is rarely presented and may...

Table 1. Percentage of patients in each risk group.

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Low</td>
<td>42.5%</td>
</tr>
<tr>
<td>Intermediate</td>
<td>35.7%</td>
</tr>
<tr>
<td>High</td>
<td>21.8%</td>
</tr>
</tbody>
</table>

POPULATION BASED ANALYSIS OF CHEMO-IMMUNOTHERAPY IN ADULT BURKITT LYMPHOMA AND LEUKAEMIA (BLL)

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Background. Adult sporadic BLL is a rare and highly aggressive malignancy. It is known to strongly express surface CD20 antigen. Recent data indicates that the outcome of adult BLL patients can be improved by the addition of anti-CD20 antibody rituximab to multi-agent chemotherapy. The population based outcome data of BLL is rarely presented and may...
Figure 1. Overall survival of all patients.

help to avoid reporting bias. Aim. The outcome of patients 18 years of age and older diagnosed with BLL. Methods. Adult BLL cases diagnosed in Lithuania (average annual population 3.54 million) in 2004-2010 were retrospectively identified through Hematology Monitoring System of Lithuania and their medical records were reviewed. BLL was diagnosed according to the World Health Organization criteria. Results. 35 adults were diagnosed with BLL (26 lymphoma and 6 leukemia). The median age was 32 years (range 18-81). 24 (70%) patients were male, 3 (9%) were HIV positive. 20 (77%) of lymphoma patients had St. Jude stage III-IV disease. The main location of lymphoma was gastrointestinal tract 11 (40%), 2 (6%) patients had neuroleukaemia. Two (6%) patients were not treated: one refused therapy and one died before treatment and 30 (94%) patients received short, intensive high-dose methotrexate, fractionated alkylyator based therapy. Rituximab 357 mg/m2 (off-label use) was administered 1 day before each chemotherapy cycle in 27 (90%) of the treated patients. Autologous stem cell transplantation was used to consolidate 5 (17%) patients. The median follow-up was 28.5 months (range: 0 - 71). Among the treated patients, 24 (80%) and 2 (7%) achieved complete and partial remission, respectively and 3 (10%) patients had primary progressive disease. 6 (19%) patients died during the first 8 months of the diagnosis: 2 were not treated, 3 deaths were due to primary disease progression and one was due to traumatic subdural hematoma. 2 year overall and progression free survival were 81% (95% CI: 62% - 91%) in all patients (Figure 1). In patients who received treatment, 2 year overall and progression free survival were 86% (95% CI: 67% - 96%). Conclusion. Our population based analysis confirms favorable outcome of all patients receiving brief intensive chemotherapy combined with anti-CD20 immunotherapy. 10% of the patients have primary refractory disease.

0961 RESULTS OF A LYMPHOBLASTIC LEUKAEMIA-LIKE CHEMOTHERAPY PROGRAMME WITH RISK-ADAPTED MEDIASTINAL IRRADIATION AND STEM CELL TRANSPLANTATION FOR ADULT PATIENTS WITH LYMPHOBLASTIC LYMPHOMA

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Background. In lymphoblastic lymphoma (LL) the role of mediastinal radiotherapy (mRT) and stem cell transplantation (SCT) remains controversial. In a risk-oriented design, we adopted a flexible treatment in which (1) patients with persistent mediastinal abnormality, evaluated by postinduction CT scan, received mRT; and patients with persistence of MRD, evaluated by molecular analysis of BM, underwent stem cell transplantation (SCT). Aims. To evaluate the clinical outcome, prognostic factors and the pattern of recurrence adopting this innovative strategy. Methods. Between 2000 and 2008, 36 B-LL and 24 T-LL untreated pts, median age 27 years (range, 16-77), M/F 17/18, 21 with mediastinal and 12 with BM involvement (≥20%) were enrolled in the NILG 99/00 protocol (Bassan, et al., Blood, 2009). The treatment included an induction/early consolidation with Ida/Vp/Asp/Cy (blocks 1-3, 5, 6, 8), HD-MTX/Ara-C (4, 7), CNS phase and mRT (Cy 24-32) for pts with pathological postinduction CT scan. Postconsolidation therapy was MRT risk oriented. MRD negativity (Mneg) was defined by low positive (<10-4) and negative determinations obtained before blocks 6 and 8, respectively. Mneg pts received maintenance, while Mpos underwent family-related/unrelated SCT (or 2-4 autologous stem-cell supported hypercycles [H-C: L-FAM/Vp/6MP]; HD-MTX/Ara-C) followed by maintenance). Pts with undefined MRD were scheduled to receive maintenance if standard risk (SR - only the pts with CD10+ B-LL), and SCT if high risk (HR-all other pts). Results. Twenty-eight pts (93%) (T-LL, n=24; B-LL, n=6) achieved CR and 2 had refractory T-LL and died. Of 21 pts with mediastinal mass, 13 (62%) achieved a CR after chemotherapy alone, whereas 6 (28.5%) required additional mRT. Eleven pts were evaluated for MFRD: 6 were Mneg and 5 Mpos. On the basis of MRD findings and clinical risk characteristics, 14 pts underwent SCT, 13 received maintenance chemotherapy, and one had local RT. Five pts relapsed. Among the 14 nonirradiated pts with T-LL, the mediastinal recurrence rate was only 7%. After a median follow-up of 3.9 years (range, 0.8-9.2+ years) 32 pts (75%) who responded were alive: 21 pts and one in 2nd CR. Postremission failure was due to recurrence (n=5, 18%) in the BM (B-LL, n=1; T-LL, n=3; 1 irradiated and 2 nonirradiated patients) or mediastinum (n=1, non-irradiated T-LL patient), and other malignancy (n=2, 7%); 1 sAML and 1 sDLBCL). The projected 5-year OS, DFS, and relapse rate were 72% (93% for B-LL and 69% for T-LL), 77% (83% for B-LL and 71% for T-LL), and 18%, respectively. The probability of OS and DFS was not significantly affected by any of examined prognostic factors, however the achievement of Mneg status was associated with a better outcome in comparison with Mpos cases (5-yrs DFS 80% vs. 60%, P=.ns). Conclusions. This programme induced high remission and survival rates, with an acceptable toxicity profile. Avoidance of mRT and SCT in patients with an early response in the mediastinum and without molecular evidence of disseminated LL limits treatment-related toxicity and warrants high cure rates in a significant proportion of patients exposed to standard chemotherapy only.

0962 THERAPY WITH 90Y IBRUTIMOMAB TIUXETAN IN RELAPSED/REFRACTORY NON-HODGKIN LYMHOHEMA. ANALYSIS OF RECENT OUTCOMES

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Introduction. Add-on treatment of follicular non-Hodgkin’s lymphoma (NHL) with 90Y ibritumomab tiuxetan (Zevalin®) has become an efficient alternative. The aim of this study is to analyze our updated information of patients treated with 90Y/ibrutimomab/tiuxetan in a prospective study according clinical practice setting and to analyze treatment outcome. Subjects and Methods. 87 relapsed/refractory lymphoma patients were included in a clinical protocol conducted by a multidisciplinary team and treated in the same centre. According the inclusion criteria, found relapsed/refractory CD20+ NHL patients with neutrophils ≥ 1.5 x 109/L, platelets ≥ 100 x 109/L, bone marrow lymphocytes CD20+ ≤ 25%. All patients received 0.5 or 0.4 mCi /kg IV (88%) of 90Y/ibrutimomab/tiuxetan and response evaluation was performed 12 weeks after. Period of study: September 2008/September 2010. Endpoints: objective response rate (ORR), time to relapse and overall survival (OS) and safety. Other clinical prognostic factors were observed to assess their possible influence upon treatment value. Results. Until September 2010, 87 patients had received treatment with 90Y/ibrutimomab/tiuxetan, and were considered to analysis: M/F 52.6%/47.4%; adult 96%, 96 years. 88% of patients enrolled in first line, 68 folll (40-86), ECOG 0-1 96.2%, previous therapy schedules 1-2 (44.8%), ≥ 2 (55.2%). The median follow-up time: 30.23 months,
**0963**

**EARLY STAGE FOLLICULAR LYMPHOMA: ROLE OF MOLECULAR MONITORING IN PATIENTS TREATED WITH LOCAL RADIOTHERAPY ± RITUXIMAB**

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**Background.** Conventional treatment of stage I-II follicular lymphoma (FL) is local radiotherapy (RT), which allows eradication of the disease in about 50% of patients. Aim. To evaluate the role of anti-CD20 MoAb and of minimal residual disease (MRD) in this setting of patients. Methods. 41 consecutive patients with a confirmed diagnosis of stage I-II FL were investigated by PCR in order to identify the presence of Bcl-2 rearranged cells in the bone marrow (BM) and/or peripheral blood (PB). All patients were treated with involved field RT (36 Gy). Subsequently, MRD was evaluated every 6 months in patients positive at baseline. Results. PCR analysis revealed Bcl-2 rearranged cells in 24/41 patients (58.5%) at presentation. After irradiation of the sole site of the disease, Bcl-2 rearranged cells disappeared in 15 of the 24 (62.5%) patients positive at baseline; in 8 (19.5%) MRD was positive, while 1 patient refused the test. After a median follow up of 50 months, 5 patients (12.2%) had a clinical relapse. MRD evaluation demonstrated that: - 17/41 Bcl-2 negative patients at the basal evaluation were not subsequently retested; only 1/17 patients had a clinical relapse (the new biopsy documented a mantle cell lymphoma). - Of the 15 patients positive at baseline and who became negative after RT, 3 have had a molecular relapse during the follow-up, leading in one case to an overt clinical relapse. - Of the 8 patients persistently Bcl-2 positive after radiotherapy, 3 had a clinical relapse. Rituximab (375 mg/m2 x 4) was administered to 5 patients with a persistently positive Bcl-2 after RT: 3 of them became Bcl-2 negative. Conclusions. Viable Bcl-2+ cells can be demonstrated in the BM and/or PB of the majority of stage I-II FL patients (despite a negative BM biopsy). Irradiation of the sole nodal/extranodal disease sites allows disappearance of Bcl-2+ cells in the majority of previously positive patients (62.5%). Pre-treatment Bcl-2 BM and/or PB evaluation has a prognostic role: no clinical relapses were observed in Bcl-2 negative cases at baseline except for one patient, relapsed as mantle cell. MRD evaluation has a prognostic role: among 32 Bcl-2 negative patients after treatment, 2 relapses (6.2%) were observed (1 relapsed as mantle cell), while among 8 Bcl-2 positive patients after treatment 3 relapses (37.5%) were observed. Prognosis of early stage FL treated with local RT ± rituximab is excellent: only 5 patients have so far relapsed at a median follow up of 50 months.

**0964**

**RITUXIMAB RETREATMENT IN PATIENTS WITH RELAPSED OR REFRACTORY B CELL NON-HODGKIN'S LYMPHOMA: PREDICTIVE FACTORS AND SAFETY**

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**Background.** Use of rituximab, a monoclonal antibody targeting CD20, is a milestone for B-cell non-Hodgkin’s lymphoma (NHL) treatment. In patients with relapsed B-cell NHL, retreatment of rituximab, with or without salvage chemotherapy, has been shown to exert promising safety and efficacy. However, a substantial proportion of patients would eventually fail by this approach. Aims. In this retrospective study, we aimed to explore factors predictive for response of rituximab retreatment and determine whether response could be translated into progression-free survival (PFS). A second objective is to re-examine the
safety and efficacy of rituximab retreatment in a hyperendemic area of chronic hepatitis B virus infection. Methods: This is a single institute study that retrospectively analysis patients with relapsed or refractory B cell NHL, who had received retreatment of rituximab either alone or in combination with salvage chemotherapy. All patients had received first-line rituximab-containing treatment. Patient’s characteristics at initial diagnosis and at relapse were collected. Response to treatment was evaluated according to the standard criteria for complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). For those who reached complete response and partial response were defined as responder. Results: A total of 54 patients who received rituximab retreatment were identified. The overall response rate to first-line rituximab-containing treatment was 83.9%, CR 27.8%, PR 61.1%. Most patients (92.5%) received rituximab retreatment in combination with salvage chemotherapy. The overall response rate of rituximab retreatment was slightly lower than the first-line treatment (61.1%) but the CR rate was similar (27.8% vs 29.6%). Median PFS and median overall survival (OS) from rituximab retreatment were not reached, with a 5-year PFS rate of 81.5% and a 5-year OS rate of 63.0%. Factors associated with better response to rituximab retreatment were high absolute lymphocyte count at retreatment ([ALC-R], defined as absolute lymphocyte count ≥1000/UL, p=0.006) and low IPI at retreatment ([IPI-R], defined as IPI ≤2, p <0.001). Both of which were significant in multivariate analysis. Incidence rate of febrile neutropenia was 38.3% but was not associated with response rate, PFS or OS. Moreover, a high incidence of herpes zoster reactivation (14.8%) and a low incidence of HBV reactivation (3.7%) were observed during rituximab retreatment. Conclusion: For patient with relapsed/refractory B cell NHL, retreatment with rituximab-containing regimen is a promising and generally tolerable salvage approach. Patients with low ALC-R and high IPI-R might predict worse response to rituximab retreatment. Febrile neutropenia and herpes zoster reactivation are the most common adverse events during or after rituximab retreatment. Acute exacerbation of hepatitis B virus infection or other opportunistic infections were uncommon. Further prospective controlled trials are needed to warrant our findings.

0965

COST-EFFECTIVENESS OF FIRST-LINE RITUXIMAB MAINTENANCE TREATMENT FOR FOLLICULAR LYMPHOMA

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2Roche Oy, Espoo, Finland

Background. Rituximab (R) and chemotherapy induction treatment has been recommended for patients with follicular lymphoma (FL). Just recently, guidelines have started to recognize the first-line R-maintenance (1LRMT) phase in patients presenting with FL in Finland. Aims. To project the lifetime health outcomes for the 1LRMT and observation, and to compare the lifetime cost-effectiveness of the 1LRMT and observation in patients presenting with FL in Finland. Methods. A probabilistic Markov state-transition model based on the PRIMA (Primary Rituximab and MAintenance) phase III trials’ 38 months first-line results and EORTC20981 phase III 60 months’ subsequent-line trial results and literature was developed in order to perform the analysis from the Finnish public health care payer perspective. The model was used to simulate patients’ transitions between first-line progression-free (PF1), PF2, progression and death states using a second order Monte Carlo simulation (2000 simulations recorded), one month cycle, and half cycle correction. Parametric extrapolation for the PRIMA PF1 results was done. The best fitting survival model was Gompertz and the maximum of 4 year treatment benefit was assumed for R in PF1 state due to the immature PRIMA data. After PF1, eligible patients were assigned for second R-maintenance based on the PRIMA results and the recent ESMO guideline for FL. For PF2, the recently published Finnish modelling results based on the EORTC20965 5-year data (maximum 5 year treatment benefit assumed) were used. Age-dependent transition to death was set equal to the larger of EORTC20965 trial or Finnish background mortality. Case-mix adjusted Finnish treatment, safety, monitoring, management and cost (excluding drug and real value, and the most affordable public drug costs (€2011; drug wastage included) were used. EQ-SD-based utilities were used. Discounting with 3% per annum was used for costs and health outcomes. The impacts of various assumptions (e.g. discounting with 0%, and PF1 function forms as Weibull, Exponential, Log Logistic, Log Normal and Gamma) were assessed. Results. The difference in survival estimates was significant for 1LRMT in comparison to observation, 1LRMT was projected to result to expected 8.56 (95%CI 7.85-9.25) quality-adjusted life-years (QALY) and 11.25 (95%CI 10.40-12.25) life-years meanwhile observation resulted to 7.18 (95%CI 6.38-8.16) QALYs and 9.61 (8.57-10.98) life-years. The incremental cost-effectiveness ratio (ICER) for 1LRMT vs. observation was €50025 per QALY gained and €6431 per life-year gained. According to the cost-effectiveness acceptability frontier, 41.6%, 96.1% and 99.9% of patients with 1LRMT were cost effective (i.e. had lower ICER than the willingness to pay threshold) at the WTP levels of €10000, €15000 and €20000 per QALY gained, respectively (Figure 1). The expected value of perfect information with highest with the ICER value of €10025 per QALY gained (€830 per patient; Figure). The relative results were robust according to the sensitivity analyses. Conclusions. 1LRMT was projected to result to significant gains in health outcomes. In addition and irrespective of the comparative assumptions, 1LRMT was a potentially cost-effective treatment option for the FL.

0966

TEMPORAL AND GEOGRAPHIC VARIATIONS OF WALDENSTRÖM’S MACROGLOBULINEMIA INCIDENCE: A LARGE POPULATION-BASED STUDY

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2Qila Hospital, Shandong University, Jinan, China
3Department of Family and Community Medicine, Baylor College of Medicine, Houston, United States of America

Background. Waldenström’s macroglobulinemia (WM) is a non-Hodgkin lymphoma (NHL) subtype. Little is known about the incidence and trends for this disease in the United States. Methods. Twenty-year data from the Surveillance, Epidemiology, and End Results (SEER) program were used for this study. SEER*Stat was used for data analysis. Results. Of the 95 797 cases of NHL diagnosed between 1988 and 2007 in nine SEER registries, 1835 (1.9%) were new cases of WM. Median age at diagnosis of WM was 73 years. The overall annual age-adjusted incidence was 0.38 per 100 000 persons per year, which increased with age, ranging from 0.03 in patients <50 years to 2.85 in patients ≥80 years. The incidence of WM was higher in men (0.54) than in women (0.27) (P < .001) and was higher in whites (0.41) than in African Americans (0.18) or other races (0.21) (P < .05). The annual percent change (APC) for the whole population was 1.01% (P > .05). The APC was 2.12% for whites (P < .05) and 0.80% (P > .05) for non-White. Significant APC increases were seen in the 70-79 age group (1.24%; P < .05) and in three geographic registries (P < .001). Conclusions. Although the overall incidence of WM remained steady over time, significant increases in incidence were seen over the past 20 years in whites, in those aged 70 to 79 years, and in three geographic registry areas.
Red cell clinical and transfusion

KNOWLEDGE DEFICITS IN BLOOD COMPONENT TRANSFUSION AMONG JUNIOR DOCTORS IN THE UNITED KINGDOM

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2Aldenbrookes’s Hospital, Cambridge, United Kingdom

Background. 2.9 million units of blood products are administered annually to approximately 400 000 patients in the UK, usually prescribed by junior doctors. Most adverse events reported are, at least partly, attributable to human error. However, blood transfusion occupies a very small part of the undergraduate curriculum and most postgraduate exams. No published data exists on the level of knowledge junior doctors have of blood product transfusion, its indications and risks. Aims. To determine knowledge among non-consultant grade doctors of the indications for, risks, compatibility, complications and broader context of blood component transfusion.

Methods. A questionnaire distributed to non-consultant, non-haematology trainee, but otherwise unselected, hospital doctors at 6 hospitals, three with medical schools and three without. The questionnaire contained 14 multiple response questions covering 1. Respondent qualifications, grade and specialty 2. Indications for transfusion of platelets, FFP and packed red cells 3. Nature and magnitude of risks associated with use of these products 4. The products themselves and issues of compatibility 5. Recognising complications of transfusion 6. National Health Service Blood and Transplant (NHSBT) and the regulatory context of blood transfusion. The responses were compared to pre-determined correct answers drawn from the annual UK Serious Hazards of Transfusion reports, official government statistics, UK transfusion guidelines, current legislation and information that could reasonably be expected to be taught to medical staff or be available in standard medical textbooks (especially ABO compatibilities). Results. 205 valid questionnaires were returned (approximately 40% response rate). No respondent correctly identified all the indications for transfusion of blood components. 65% would inappropriately use red cell transfusion to promote wound healing. 80% would use FFP to treat major haemorrhage due to warfarin, an inferior approach, 85% overestimated the risk of death from blood component transfusion by at least 50 times, 7% thought contracting HIV from blood component transfusion was as likely as dying in a car accident. 30% identified imaginary complications of transfusion, such as acute psychosis or transfusion associated acute liver disease (TaLiD), as real while 30% did not identify RhD+ blood to an RhD- female recipient of child bearing age, ABO incompatibility of AB blood to an O recipient as dangerous while 7% thought contracting human error. However, blood transfusion occupies a very small part of the undergraduate curriculum and most postgraduate exams. No published data exists on the level of knowledge junior doctors have of blood product transfusion, its indications and risks. Aims. To determine knowledge among non-consultant grade doctors of the indications for, risks, compatibility, complications and broader context of blood component transfusion.

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Table 1. Selected knowledge deficits amongst junior doctors.

<table>
<thead>
<tr>
<th>Deficit</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>Inappropriate use of red cell transfusion for wound healing</td>
<td>65%</td>
</tr>
<tr>
<td>Use of FFP for major haemorrhage due to warfarin</td>
<td>80%</td>
</tr>
<tr>
<td>Overestimation of death risk from blood component transfusion</td>
<td>85%</td>
</tr>
<tr>
<td>Inappropriately using FFP for major haemorrhage</td>
<td>7%</td>
</tr>
<tr>
<td>Not identifying RhD+ blood for RhD- recipient</td>
<td>30%</td>
</tr>
<tr>
<td>ABO incompatibility of AB blood for O recipient</td>
<td>30%</td>
</tr>
<tr>
<td>Recognising complications of transfusion</td>
<td>7%</td>
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<tr>
<td>National Health Service Blood and Transplant (NHSBT) and regulatory context</td>
<td>65%</td>
</tr>
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London, United Kingdom, June 9 – 12, 2011

KINETICS OF CLOTTING FACTORS INACTIVATION DURING PASTEURIZATION AS APPLIED TO FLEBOGAMMA® AND FLEBOGAMMA® DIF MANUFACTURING PROCESSES

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Instituto Grifols S.A., Parets del Vallès, Barcelona, Spain

Background. An increased rate of thromboembolic adverse events associated with the use of an IVIG from a specific manufacturer has been ascribed to increased residual FXa, and possibly other impurities, in that product. Pasteurization is a process with demonstrated capacity to inactivate clotting enzymes during IVIG production (José et al. WebmedCentral Immunotherapy 2010;1(12):WMC001425. Available at: www.webmedcentral.com/wmcpdf/Article_WMC001425.pdf). Aims. In this work we describe the kinetics of Pasteurization-induced inactivation of procoagulant activity, in the conditions applied during the production Flebogamma® and Flebogamma® DIF; two pasteurized IVIGs from Grifols. Methods. Plasma fraction (P) II-III derived samples, containing artificially activated clotting factors, were spiked 1/5, 1/10, 1/20 (v/v) in samples taken from the IVIG manufacturing materials before acid pH treatment (4.5h, pH 4.0, 37°C) or before Pasteurization treatment (10h, 60°C) were applied to the mixtures. The following activities markers were studied: non-activated partial thromboplastin time (NaPTT) with platelet poor plasma (PPP, either neat or after dilution in the assay buffer) or with FXI deficient (FXIdef) plasma, PKA, “kallikrein-like” activities, thrombin generation (TGT) standard test or with FXIdef plasma, and FXI antigen (FXI:Ag, ELISA). The Pasteurization treatment (10h, 60°C) was also assessed at different time points (0, 0.5, 2, 5 and 10 h) in 1/5 (v/v) spiked samples and in non-spiked controls as well as in equivalent samples spiked with 1 or 0.1 nM FXIa. Results. Samples of industrial materials before acid pH treatment or before Pasteurization spiked with artificially activated FrII-III derived samples showed positive results when assayed for coagulation markers in all tests performed (i.e., in the spike 1/5 samples values ranged 24-77 s for NaPTT-PPP neat, 43-155 s for NaPTT-PPP diluted, 53 - 114 IU/ml for PKA, 0.015-0.038 AU/min for “kallikrein-like”, 89 s for standard TGT, 351-502 s for FXIdef plasma TGT). With the exception of PKA, which was significantly reduced to <2 IU/ml, levels of markers in all the spiked mixtures remained practically unchanged after acid pH treatment even at the lowest spike proportion (1/20), thus pointing out a very limited effect of this treatment on coagulation factor inactivation. In contrast, Pasteurization rendered the spiked mixtures negative for all assays even at the highest spike proportion (1/5). Moreover, after only 2h of Pasteurization, activation markers were already negative for NaPTT -both tests-, TGT -both tests-, PKA and “kallikrein like” tests, while after 5 h Pasteurization thrombin generation showed a reduction of 80-90%, being undetectable after 5 hours. Conclusion. Even after only 2h of treatment, Pasteurization effectively inactivated relevant concentrations of procoagulant activities in artificially spiked FrII-III samples, higher than those found in the real Flebogamma® and Flebogamma® DIF production conditions. Acid pH treatment showed a very limited capacity to separate or inactivate these compounds.

DOES FIBRINOGEN CONCENTRATE REDUCE BLOOD PRODUCTS USE IN MAJOR OBSTETRIC HAEMORRHAGE?

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Background. In July 2009, the Irish Blood Transfusion Service (IBTS) replaced cryoprecipitate with fibrinogen concentrate with the aim of reducing the potential risk of pathogen transmission. Fibrinogen concentrate appears to have similar efficacy in treatment of major haemorrhage but there is limited data about its use in Major Obstetric Haemorrhage (MOH). Aims. The aim of this study was to assess the impact of this change on estimated blood loss (EBL) and blood product use in MOH. Materials and Method. Prospective detailed audit of MOH began at our institution in January 2009. Cases are defined by EBL of 2.5 litres, transfusion of 5 or more units of Red Cell Concentrate (RCC) or treatment of a coagulopathy. The EBL and the use of blood products were compared between those that sustained an MOH with associated hy- pofibrinogenemia before and after cryoprecipitate was replaced with fibrinogen concentrate. Results. 59 cases of MOH were identified in 2 years (3.3/1000 deliveries). 29 required treatment for hypofibrinogen-
emia; Cryoprecipitate (14) and Fibrinogen concentrate (15). The two groups were similar in age, parity, ethnicity and gestation at delivery. The main cause of bleeding was uterine atony followed by retained placental tissue. Medical and surgical management were similar among the two groups. Haemostasis was achieved in all cases. Minimum serum fibrinogen level recorded was 0.19g/l. The estimated blood loss was found to be higher in the cryoprecipitate group (mean+-SD=5.1+/-4 versus 260.6+/-260.6). The use of red cells and Octaplas were similarly higher in the cryoprecipitate group, however there was no difference in the use of platelets among the two groups. Conclusion. The replacement of cryoprecipitate with Fibrinogen concentrate in MOH was associated with a reduction in EBL and a reduction in use of RCC and Octaplas. These cases, however, were diverse and complex and fibrinogen replacement was only one factor in the overall management but replacement with a small volume bolus that can be administered rapidly without thawing may facilitate more rapid correction of coagulopathy and earlier haemostasis.

0970
EXPERIENCE IN THE MANAGEMENT OF LOOKBACKS IN A TERTIARY HOSPITAL TRANSFUSION SERVICE
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2Centro Regional de Transfusión, Madrid, Spain

Background. Donor selection and the use of serological and nucleic acid testing in blood donations have greatly reduced the infectious window for several transfusion-transmitted pathogens; however, this window has not been totally eliminated. Lookback programs involve identification and notification of components collected from donors that are later found to be reactive in specific serological tests. This strategy is supposed to prevent progression of disease in a transfusion recipient or to the patient’s physician. The regional transfusion centre of the recipient directly and if it is not possible, we send a letter to the recipient’s last known status (living or dead). If the recipient is identified, the hospital information system is searched to determine whether it might be necessary to change our protocol in the future.

Table 1.

<table>
<thead>
<tr>
<th>YEAR</th>
<th>PRODUCT</th>
<th>PLATELETS</th>
<th>RED CELLS</th>
<th>WHOLE BLOOD</th>
<th>PLASMA</th>
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<tbody>
<tr>
<td>2009</td>
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Figure 1.
and the rate of reports during the 2006-2009 period have pointed to an increase in all haemovigilance systems analyzed, showing a growing awareness of the need to improve transfusion safety. Globally considered, the rate of reports in Spain and the UK are similar, highlighting a positive growth of the Spanish haemovigilance system. The number of reports on TRALI have grown in all systems, beeinig higher in Spain which constitutes the system that controls transfusion policies of both countries. Haemovigilance systems provide a mechanism for monitoring the transfusion activity that allow comparisons between countries and help to develop policies to make blood as safe as possible.

0972 ADVANCED RED BLOOD CELL INDICES AND IRON STATUS AS COMPLEMENTARY FACTORS IN THE MANAGEMENT OF REGULAR BLOOD DONORS
M Sammelrock, R Raggam, HJ Semmelrock, F Prueller, K Amrein, G Lanzer, E Rohde

Background/aims. Frequent blood donations may lead to depletion of body iron stores resulting in development of anemia. Currently, screening for iron deficiency (ID) is done by determination of haemoglobin concentration (Hb) prior to each donation. Hb measurement has limitations due to the late response in case of an iron deficient state. Several methods for early detection of iron deficiency are available but lack practicability. We therefore evaluated the association of iron status with advanced red blood cell (RBC) indices in regular whole blood donors. Methods. In a prospective study, the iron status including serum ferritin and soluble transferrin receptor (sTfR) was tested in 1308 healthy blood donors after written informed consent. The ferritin index is representing the most relevant marker for ID and was calculated as the ratio of sTfR to the logarithm of ferritin. Full RBC count including percentage of hypochromic mature erythrocytes (%HYPOm) and reticulocyte haemoglobin content (CHR) was determined on the automated haematology system Advia 2120i (Siemens Healthcare Diagnostics). Gender matched study populations were assessed for signs of ID and impaired haemoglobinisation. The areas under the receiver operating characteristic (ROC) curves were calculated using CHr and %HYPOm to assess their practicability in ID diagnosis. Results. Cut-off values to detect an ID were generated by evaluating the 97.5th percentile of the ferritin index obtained in controls (male: 0.960, female: 1.621 donors). Considering these cut-offs, the highest percentage of ID (54.5%) was obtained in male donors with the highest allowed donation frequency (6 times per year). Interestingly female donors displayed the highest rate of ID already after three donations per year (24.7%), and ID rate was not higher after further donations. Among these iron deficient donors reduced levels of CHr and increased rates of %HYPOm were observed. Using CHr as a marker for ID, areas under the ROC curves were 0.790 (m) and 0.907 (f). When %HYPOm was used, areas under the ROC curves were 0.808 (m) and 0.922 (f). Conclusions. A strong correlation between iron status and RBC indices was not found. However, measurement of RBC indices allows for an early estimation of impairment in red blood cell haemoglobinisation, especially while donors may show normal haemoglobin values in absence of clinical signs and symptoms for ID. Additional testing of CHr and %HYPOm is feasible for routine screening of regular whole blood donors to better prevent the development of ID based anemia at a very early stage.

0973 NON-ABO RED BLOOD CELL ALLOANTIBODIES AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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University Hospital Basel, Basel, Switzerland

Background. The appearance of alloimmune hemolytic anemia (AHA) due to anti-A and anti-B antibodies in patients undergoing a major or minor ABO-incompatible allogeneic HSCT is one of the most common and dangerous immunohematological complication. Less frequently, other red blood cell (RBC) antigen systems have been implicated in the development of AHA. Although the hemolytic complications following ABO-incompatible allogeneic HSCT have been investigated by several authors, the development of alloantibodies against RBC antigens other than ABO has been less well investigated. Aims. We were therefore interested to examine in a single center study the presence of non-ABO RBC alloantibodies in patients treated with allogeneic HSCT between 2006 and 2009. Methods. This retrospective cohort study is based on standardized, prospectively collected clinical data from the stem cell transplant unit of the University Hospital Basel, Switzerland, and serological tests from the database of the Blood Bank Basel, Switzerland. Between January 1996 and September 2009, 514 adult patients and 73 children received 604 respectively 85 HSCT. Results. In the first two years after allogeneic HSCT the cumulative incidence for the development of non-ABO RBC alloantibodies was 7.27% (95% CI 7.25-7.29%) in adult patients respectively 14.15% (95% CI 13.87-14.43) in children. The most common detected alloantibodies were Anti-E and Anti-LuA. In all the patients in whom antibody specificity was identified, the antibody was directed against RBC antigens absent in donor or recipient’s RBCs. However in 43 (74% of the patients with alloantibodies) respectively 11 (19%) patients the alloantibody was directed against an antigen present in the transfused RBC units respectively platelet (PLT) units. In the remaining 7% of the patients the causes of alloimmunization remain unclear. The mean time between transplant and antibody detection was 18 days (range 1-2239 days), and the most alloantibodies were detected in the first 30 days after allogeneic HSCT (s, Figure 1). In the univariate analysis, CoVHD prophylaxis, conditioning regimen, and number of transfused RBC units were associated with a higher risk of development of alloantibodies. After stepwise introduction in the multivariate model, the number of RBC units remained the only variable that significantly influenced the formation of non-ABO RBC alloantibodies. In fourteen patients (24% of the patients with alloantibodies) there was evidence of hemolysis after antibody detection; two of these patients developed a documented severe immune hemolytic anemia in the early post transplant period and died. Conclusions. To the best of our knowledge this is the largest cohort study describing the development of non-ABO RBC antibodies after allogeneic HSCT. In our analyze, the cumulative incidence of alloantibodies formation was with 7% in adult patients and 14% in children higher in comparison with those reported by other authors. The only risk factor for developing non-ABO RBC antibodies was the amount of transfused RBC units. Virtually all the antibodies resulted following blood products (RBC and PLT) positive for the respective antigen; no antibodies emerged in an antigen-mismatched situation between donor and recipient. However, the most common alloantibodies detected, Anti-E and Anti-LuA, are potentially naturally occurring antibodies, not necessarily due to transfusion.

0974 AUDIT OF MEAN ANNUAL PRE-TRANSFUSION HB LEVEL OF PATIENTS WITH B THALASSEMAIA MAJOR AT THALASSEMAIA CENTRE
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Background. Thalassemia center transfusion guidelines assess the efficiency of the blood transfusion program implemented at the centre through maintaining the mean annual pre-transfusion HB ≥9.5gm/dl. This is essential in the management of B Thalassemia major patients to improve wellbeing and overall outcome. In April 2009, pre-storage leukocyte depleted packed RBCs were introduced while bedside blood
graphs were used previously. Aims. To evaluate the mean annual pre-
transfusion Hb level of patients with B Thalassemia major transfused
at the center and the impact of pre-storage leukocyte depleted blood
on the mean annual pre-transfusion Hb. Methods. This was a retrospec-
tive audit where pre-transfusion Hb was obtained form Oct-2008 till
end of Sep 2009 by reviewing patients’ files. Random sample of 100 B
Thalassemia major patients on regular blood transfusion every 3-4
weeks at thalassemia centre were selected. Patients with increased risk
at the center and the impact of pre-storage leukocyte depleted blood
were not available so the audit has exposed the importance of provision of these bags to
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at the center and the impact of pre-storage leukocyte depleted blood
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with a tendency towards higher pre test probability, but drop in percentage of positive results which raises the possibility of scores being skewed towards higher probability. We aim to overcome this by screening all requests and communicating with requesting team. Given the high percentage of negative results we aim to introduce a sensitive rapid gel agglutination card technique as a screening method subsequently confirming positive results with an IgG specific ELISA including confirmation with heparin inhibition. Given the current workload we hypothesize that further improved communication we will reduce the number of inappropriate requests and introduction of rapid screening will significantly reduce staff time and cost. We aim to re-audit once the above are implemented.

0977

COMPARISON OF IN VITRO EFFECTS OBTAINED BY COLLAGEN STIMULATION OF FRESH WHOLE BLOOD AND RECONSTITUTED WHOLE BLOOD

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Background. The use of whole blood (WB) has nearly completely been replaced by blood component therapy in transfusion medicine. However, in situations where blood components are not available or affordable, i.e. military conflict or major catastrophes, enlarged documentation of the quality of fresh WB is warranted. Also, there is an increasing debate regarding the storage lesion of blood component, e.g. reduced quality of both stored red blood cell (RBC) concentrates and platelets. Even regarding fresh frozen plasma, there is an issue because the thawing time may be critical in massive bleedings - and the concept of pre-thawed plasma has been introduced, although debated. Aims. Based on these considerations we performed a study to compare: (i) in vitro responses to collagen stimulation in fresh WB, with (ii) responses obtained by stimulation of reconstituted WB of different compositions. Furthermore, we investigated potential varying in responses between age of RBC and the platelets in the units. Methods. Nine groups of reconstituted WB with different compositions of platelets, RBCs, and plasma were compared (Table). Platelets stored for 1, 3, and 5 days were combined with RBC stored for 0-4 days, 12-16 days, and 26-35 days. Platelet concentrations for all samples were determined by the impedance method. Platelet aggregation was expressed as percentage of single platelet disappearance (SPD). Thrombelastography (TEG) was performed on fresh WB samples and reconstituted WB samples, and the effects of storage time on fresh WB were tested to preclude this as a source of error. In vitro thrombin generation was estimated by thrombin-antithrombin (TAT), quantified by standard enzyme-linked immunosorbent assay (ELISA). Results. A significant decrease (P<0.001) in SPD between unstimulated samples and samples stimulated with collagen was seen in all groups, with exception of E, F, and I. SPD was found to be lowest in WB and the groups containing the oldest RBC- and platelet concentrates. Regarding the stimulated samples, significant differences were found between WB vs. group A and B, in addition to group A vs. E, F, and I, and group B vs. F and I. Results from TAT complexes showed that thrombin generation decreased according to storage time in stimulated reconstituted WB samples, corresponding to the results obtained from the SPD. TAT complexes were significantly lower in fresh WB as compared to group A and B after collagen stimulation. None of the groups show significant deviations from standard TEG parameters. Conclusion. The results show that storage time of both RBCs and platelets may influence the effects of blood component stimulation. Therefore, WB over reconstituted WB will result in decreased SPD. Further study to clarify the clinical relevance of these effects are warranted.

Table 1. The composition of the groups - with SPD.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBCs</th>
<th>Platelets</th>
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<tbody>
<tr>
<td>A</td>
<td>Fresh</td>
<td>1 day</td>
</tr>
<tr>
<td>B</td>
<td>Fresh</td>
<td>3 days</td>
</tr>
<tr>
<td>C</td>
<td>Fresh</td>
<td>5 days</td>
</tr>
<tr>
<td>D</td>
<td>1 day</td>
<td>Fresh</td>
</tr>
<tr>
<td>E</td>
<td>1 day</td>
<td>3 days</td>
</tr>
<tr>
<td>F</td>
<td>1 day</td>
<td>5 days</td>
</tr>
<tr>
<td>G</td>
<td>3 days</td>
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</tr>
<tr>
<td>H</td>
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<tr>
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0978

FREQUENT USE OF BLOOD TRANSFUSIONS IN CURRENT TREATMENT PRACTICE FOR CHEMOTHERAPY-INDUCED ANEMIA COUNTERACTS TREATMENT RECOMMENDATIONS AIMING FOR LESS TRANSFUSIONS

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Background. Patients with cancer frequently experience chemotherapy-induced anaemia (CIA) and iron deficiency (ID). Clinical evidence suggests that IV iron supplementation is a cost-effective treatment for CIA and ID. We aimed to assess how many patients with CIA, and iron deficiency were receiving blood transfusions, and explore reasons for their regular use in current treatment practice. Methods. Eligible onco-hematologists were recruited at random and completed records on their last five patients treated for CIA in two waves: Wave one (France, Germany, Spain, Switzerland, UK, Jun-Oct 2009), Wave two (Austria, Italy, Netherlands, Sweden, Aug-Nov 2010). Overall results are presented as median between [range] across countries and detailed results of wave two countries as % of all cases and [range] across countries. Results. 375 physicians recorded 1730 cases of CIA, 58% [38-77%] in patients with solid tumors of whom 52% [30-68%] had metastatic disease. At diagnosis of anemia, 14% [5-28%] of patients presented with severe anemia (Hb <8 g/dL) and 20% [8-41%] of ferritin-tested patients with absolute ID (ferritin <30 μg/L), 52% [11-93%] received a transfusion at some stage and 73% [15-100%] received an ESA. Iron was given to 22% [11-61%] and thereof only 19% [4-77%] received iron supplements. Of all patients receiving transfusions, 60% [11-100%] were given a combination with ESA and 27% [7-51%] with iron. Detailed questions regarding the use of blood transfusions by physicians participating in Wave two (131 physicians, 651 cases) show that blood transfusions were given as regular treatment in 76% [48-85%] of cases and except for Italy (44%) only rarely as emergency treatment (11-18%; 15% of all cases). In 45% [26-55%], blood transfusions were given at least once every three months and in 3% [0-7%] even weekly or more often. Over a 12-month period prior to the survey, transfused patients received 5 units [2-6 units] blood concentrates. Among the options in the questionnaire, ‘easily available’ (47% [15-62%]) and ‘uncomplicated use’ (34% [0-52%]) were most commonly selected as reasons for administration of blood transfusions. Further reasons were that anemia was not controlled by ESAs alone (29% [14-69%]) or by the given iron treatment (24% [0-39%]). In 82% [77-84%] of these iron-treated patients, oral iron was administered at high total doses of 16.6g [10.6-32.8g] given over 12 weeks (9-20 weeks). Conclusions. More than half (52%) of the patients treated for CIA received blood transfusions while ESAs were given to 73% and iron (mostly oral) to only 22% of patients. Frequently, transfusions were given on a regular basis and not only as a rescue therapy reflecting the suboptimal results obtained with ESAs alone or IV iron supplementation. As IV iron supplementation of ESAs improves hematologic response, awareness of this option should be increased in order to minimize the use of red blood cell transfusions.
Background. Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening disease, characterized by systemic thrombotic microangiopathy (TMA), which is caused by chronic uncontrolled terminal complement activation. Systemic TMA, presenting with end-organ damage, hemolytic anemia and platelet consumption and leads to progressive renal disease, multi-organ damage and, ultimately death. Importantly, many patients with aHUS receive chronic plasma exchange/infusion, but despite this, still continue to have persistent TMA and poor clinical outcomes, and up to 60% of patients develop end-stage renal disease or die within 1 year of diagnosis. Aims. In a phase II trial, we evaluated the efficacy and safety of eculizumab, a terminal complement inhibitor, in plasma exchange/infusion-resistant aHUS patients. Methods. This is a 26-week, controlled, open-label, single-arm trial. Patients were enrolled who were ≥12 years and had plasma exchange/infusion-resistant aHUS (persistent TMA despite ≥4 plasma exchange/infusion sessions 1 week before screening). The eculizumab intravenous dosing schedule was 900mg/week for 4 weeks, 1200mg at week 5, then 1200mg q2 weeks. All patients received a meningococcal vaccine. The primary endpoint was the change in platelet count (a measure of TMA) over 26 weeks. Secondary endpoints included TMA event-free status (≥12 weeks of stable platelet count, no plasma exchange/infusion and no new dialysis), TMA intervention rate (number of plasma and new dialysis events/patient/day), renal function, pharmacokinetics/pharmacodynamics (PK/PD), health-related quality of life (HRQoL) measured by ED-5D and safety. Results. A total of 17 patients were enrolled; 15 received eculizumab for the entire 26-week period. Median age was 28 years (range 17-68 years), 29% were males, 76% had an identified complement regulatory factor mutation, 41% had a kidney transplant, and 29% were on dialysis prior to eculizumab. Following eculizumab treatment, platelet count increased from baseline to week 26 by a highly statistically significant; mean change from baseline to week 26 was 0.33±0.09 (p<0.0001). The most frequently reported adverse events were headache, anemia and diarrhea (all mild-moderate in severity). Conclusions. In summary, the primary and secondary endpoints were achieved with high clinical and statistical significance. Eculizumab prevented TMA, restored renal function, removed the need for plasma exchange/infusion and improved HRQoL. Eculizumab was also well tolerated.
ECLIPSE: A FRENCH STUDY CONCERNING THE DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)

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Aim. PNH is a rare life-threatening disease with prevalence between 7.8 and 15.9 cases/million. Diagnosis is difficult and often delayed because of clinical polymorphisms. ECLIPSE is a French study aimed to evaluate the delay between the onset of PNH symptoms and diagnosis, to identify the clinical signs leading to diagnosis and to determine which medical specialists are seen first by PNH patients. Patients and Methods. 4920 physicians were asked to participate: 992 hematologists, 1638 internists, 1155 gastroenterologists, 697 nephrologists, 438 neoplastic physicians. Physicians were divided into 3 groups: (A) having diagnosed PNH at least once, (B) having suspected a PNH without having confirmed the diagnosis, (C) neither having suspected nor diagnosed PNH. Results. 528 physicians accepted to participate in the study (overall response rate: 10.7%). 507 answers were analyzed. Among the 507 physicians, 108 (21%) belonged to group A, 215 (42%) to group B, and 185 (37%) (CI95% - 35.4 : 40.6%) to group C. In group A, clinical signs and symptoms leading to diagnosis were: pancytopenia (44%), anemia (37%), hemolysis (23%), peripheral venous thrombosis (18%), hepatic vein thrombosis (14%) and hemoglobinuria (14%). Clinical situations raising the suspicion for diagnosis within group A physicians were: unexplained thrombosis (36%), hemoglobinuria (84%), aplastic anemia (83%), Coombs negative anemia (80%), cytopenias (71%). Physicians were also asked to describe the circumstances of their latest PNH diagnosis. The patient was referred to the physician by the Emergency (23%), a haematologist (23%) or internist (21%). Most frequent functional symptoms were: fatigue (39%), anemia (24%), abdominal pain (20%) and thrombosis (14%). 7% of patients were asymptomatic. PNH diagnosis was confirmed in a mean time of 9.3±11.46 months after the onset of symptoms, and a maximum delay between first symptoms and diagnosis being 60 months. Biological signs raising the suspicion for a PNH were: anemia (80%), increased LDH (60%), increased bilirubin (44%), thrombocytopenia (41%) and/or neutropenia (28%). Confirmation of PNH diagnosis was made by flow cytometry in 87% of the cases. Among the 218 physicians belonging to group B, 50% had suspected at least 5 times PNH without confirmation. Clinical and biological signs prompting group B physician to suspect PNH were: Coombs negative anemia (48%), pancytopenia (42%) and/or aplastic anemia (58%), myelodysplastic syndrome (18%), hemoglobinuria (15.5%), increased LDH associated with venous or arterial thrombosis (15%), abdominal pain (14%), dark urine (12%) or jaundice (11%). 186 physicians belonged to group C. 6.5% of physicians had never heard about PNH. Conclusions. PNH was mainly diagnosed by hematologists. Frequent symptoms leading to diagnosis were unexplained thrombosis, hemoglobinuria, Coombs negative anemia, aplastic anemia, cytopenias and myelodysplastic syndrome. Flow cytometry, the gold standard for PNH testing, was only used in 87% of cases. Diagnosis was usually delayed with a maximum of 5 years between onset of symptoms and diagnosis. Fatigue and abdominal pain were commonly reported and should therefore be more routinely assessed.

HIGH PREVALENCE OF IRON DEFICIENCY ACROSS DIFFERENT TUMORS CORRELATES WITH ANEMIA, INCREASES DURING CANCER TREATMENT AND IS ASSOCIATED WITH POOR PERFORMANCE STATUS

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2Vifor Pharma, Glattbrugg, Switzerland

Background. Iron deficiency (ID) with or without anemia affects physical function and quality of life. In patients with cancer or chronic inflammatory diseases, impaired iron utilization can limit effective erythropoiesis. Presently, only limited data on iron status and its relation to hemoglobin levels, clinical status and antineoplastic cancer treatment are available. Aim. This observational study aimed to assess the prevalence of ID and its relation to hemoglobin levels, performance status, cancer type, and disease and treatment status in a large cohort of unselected cancer patients with different cancer types. Patients and Methods. 1528 cancer patients that presented consecutively from October 2009 to January 2010 at our center were evaluated for ECOG performance status, cancer type, stage at initial cancer diagnosis, status of disease at evaluation (CR/no evidence, persistent, or progressive disease) and for time of last treatment. Further, iron parameters and hemoglobin levels were assessed. The following definitions were applied: anemia (hemoglobin, Hb <12 g/dL), ID (transferrin saturation, TSAT <20%), absolute ID (AID, serum ferritin <30 ng/mL or TSAT <10% if ferritin was not available) and functional ID (FID, ferritin ≥30 ng/mL or TSAT >10%). Results. 1053 patients presented with solid tumors and thereof 48.2% had metastatic disease. Anemia and ID were most prevalent in pancreatic cancer (50%), Table. Anemia was more prevalent in patients with stage IV (42.2%) compared to those with stage I/II or stage III disease (28.8%). The prevalence of FID increased with stage (29.1%, 35.3% and 45.6% in stage I/II, stage III and stage IV respectively) whereas AID was comparable across stages (6.7%, 10.0%, 7.7%, respectively). The prevalence of anemia and FID correlated with worse ECOG performance status (ECOG 0-1: 29.3% anemic, 36.1% FID; ECOG 2-4: 61.1% anemic, 52.8% FID). A concomitantly higher prevalence of anemia and FID was also observed in patients receiving antinecancer treatment within 12 weeks prior to evaluation compared to patients without therapy (anemia 48.6 vs. 32.4%; FID 42.8 vs. 38.4%). Among patients who have received treatment more than 15 weeks prior to evaluation, anemia, FID and AID were less prevalent (17.1%, 35.9%, 5.8%, respectively). Persistent and progressive disease at time of evaluation was associated with high rates of anemia and ID (47.1% and 56.8%, respectively). Notably, although 77.8% of patients in complete remission achieved normal Hb levels, 36.4% remained iron deficient. Conclusion. This analysis shows a high prevalence of iron deficiency, in particular functional iron deficiency, across different tumor types. The prevalence of ID correlates with the prevalence of anemia and progression of the disease. ID is also associated with worse performance status. In patients receiving antinecancer treatment, the higher prevalence of anemia is paralleled by a higher prevalence of ID.

FERRIC CARBOXYMALTOSE FOR THE CORRECTION OF CANCER AND CHEMOTHERAPY-ASSOCIATED ANEMIA IN CLINICAL PRACTICE

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Background. Iron and iron deficiency are frequent complications in cancer patients. Intravenous iron as a supplement to erythropoiesis-stimulating agents (ESA) has been shown to improve hemoglobin (Hb) levels, reduce the number of blood transfusions and decrease the need for ESA in anemic cancer patients. Aim. This observational study evaluated the effectiveness and tolerability of ferric carboxymaltose (FCM) in routine treatment of anemia in cancer patients. Methods. Adult cancer patients with anemia were enrolled from Dec 2008 - Jul 2010 at 68
German hematology/oncology practices and observed until Week 12 (+2) post inclusion or the termination visit. FCM was administered without restriction on dosing, concomitant use of ESAs or transfusions. Of 639 enrolled patients, 364 with available Hb measurements at baseline and at least one follow-up visit were analyzed for Hb increase (primary endpoint), 420 with available Hb measurements at baseline for secondary effectiveness parameters and 619 who received at least one FCM dose for safety. Effectiveness analyses included stratification by baseline Hb-, ferritin levels and subgroups who received FCM only or FCM and ESAs. Patients who received blood transfusions were censored prior to the transfusion. Data are shown as median values (25%, 75% quartiles) unless otherwise stated. Results. Most patients in the effec-
tiveness population (female 54.8%, 67 years [58, 73]) presented with solid tumors (91.2% total; 25.2% breast, 19.8% colorectal, 8.8% stomach), of which 61.0% were metastasized. 74.3% received con-
comitant cytotoxic chemotherapy and 24.3% had at least one anemia treatment during four weeks prior to study inclusion (15.1% transfusions, 8.3% ESAs, 4.0% i.v. or oral iron, 0.7% others). Median baseline Hb in the effectiveness population was 10.0g/dL (9.1, 10.6), 37.5% of tested patients had a ferritin ≥100nmL and 75.6% a transferrin saturation <20%. Median total iron dose per patient was 1000mg (600, 1500). Median increase in Hb levels was 1.4g/dL (0.2, 2.3) in the overall population, 1.4g/dL (0.2, 2.3) in patients who received FCM only, 1.6g/dL (0.7, 2.4) in patients who received FCM plus ESAs, and 1.4g/dL (0.3, 2.3) in patients censored for transfusions during the study. Hb levels improved steadily after the first FCM administration. From Week 5 onwards, mean Hb levels remained stable in the range of 11-15g/dL and were comparable between patients treated with FCM alone or concomitant ESAs as well as in patients with mild (baseline Hb 10-11g/dL) and moderate-to-severe (baseline Hb <10g/dL) anemia. Patients with baseline ferritin levels <100ng/mL achieved Hb levels >11g/dL earlier (Week 3–4) than those with baseline ferritin ≥100ng/mL (Week 7). FCM was well tolerated. Possibly or probably drug-related adverse events (AEs), mainly nausea and diarrhea, were reported for 2.4% (n=14) of patients. Three serious AEs (SAEs) comprised one fatal case after a possibly related respiratory insufficiency and two unlikely related events of tachycardia and dyspnea. Conclusions. FCM effectively improved and stabilized Hb levels of anemic cancer patients at 11-15g/dL in routine clinical practice, even without concomitant ESAs. Furthermore, the results of this observational study suggest that FCM can provide benefit to cancer patients independent of baseline Hb levels.

**Figure 1. Median Hb over time.**

**Table 1.***

<table>
<thead>
<tr>
<th>Impact of different Hb levels at DA initiation on transfusion rate and cost of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA (g/L)</td>
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<tr>
<td>----------</td>
</tr>
<tr>
<td>&lt;9</td>
</tr>
<tr>
<td>9-10</td>
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<tr>
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**0984 THE EFFECT OF HEMOGLOBIN LEVEL AT DARBEPOETIN ALFA INITIATION ON TRANSFUSION REDUCTION AND POTENTIAL COST-SAVING IMPACT IN THE TREATMENT OF CHEMOTHERAPY-INDUCED ANEMIA**

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Background. Patients with cancer who receive chemotherapy may develop chemotherapy-induced anemia (CIA). Efficacy of erythropoiesis-stimulating agents (ESAs) in reducing the incidence of transfusions and increasing hemoglobin (Hb) levels has been demonstrated (Aapro, The Oncologist. 2005;10 (Suppl 3):S3-8). According to current guidelines in the US and Europe, ESA treatment should be initiated as the Hb level approaches either 10 g/dL (NCCN, USA) or between 9-11 g/dL (EORTC, Europe) in patients with anemia-related symptoms. Aims. The objective of this study was to understand the impact of Hb level at the start of darbeepoetin alfa (DA) treatment on transfusion reduction and to identify the cost-saving impact of this reduction on CIA treatment costs. Methods. Two separate systematic literature reviews were performed. We conducted a systematic review of the clinical literature in PubMed, as well as ASCO, ASH, ESMO, ESMO/ECOCO, and EHA conference abstract databases between 2006 and 2010. Search terms included “DA” and “CIA”. DA is an ESA with dosing regimens up to every three weeks. A systematic review of economic studies on cost-of-transfusion was performed using a PubMed search from 2000-2010. Reference lists of retrieved studies from this review were scanned to identify additional articles. Mesh terms included “anemia/economics”. Results. Eight studies were retrieved from the clinical literature review; six full-text articles and two conference ab-
stracts. Six were based on clinical trials whereas two were based on ob-servational studies. Despite the differences in baseline patient character-
istics, length of the studies and analytical techniques, the need for transfusions decreased across all studies when DA initiation occurred at higher Hb levels. Twenty-one studies met the inclusion criteria for the economic literature review. These studies included that the cost of one unit of red blood cell (RBC) transfusion ranged from €240-414 in Europe, USD$107-529 in USA, £90-402 in UK, CAN$280-456 in Canada and AUS$143 in Australia. Few studies reported actual number of units transfused. When the number of transfusions was reported, more than one unit was usually transfused in the majority of patients. To estimate potential savings in CIA treatment associated with Hb level at the time of DA initiation, the difference in transfusion rates was multiplied by the midpoint of transfusion cost range in Europe (€527). An illustrative example using two of the eight identified clinical studies (latest studies) is presented in Table 1. Potential cost savings ranged from €59-3,548 for every 10 patients treated. We performed the same analysis using the remainder of identified studies and found the same trend of cost-savings (data not shown). Conclusions. The find-
ings of the clinical systematic review suggest that transfusion incidence decreases with higher Hb levels at DA initiation. Cost of transfusions was found to vary from country to country and depended on cost items included (e.g. direct costs, indirect costs). Our findings suggest that the resulting cost-savings depends on the number of RBC units transfused and cost items included. Initiation of DA according to guidelines is important in terms of reducing the number of transfusions as well as the potential cost-saving impact on CIA treatment.
DONOR ALLELE (GT)16 IN THE PROMOTER/ENHANCER REGION POLYMORPHISM OF FOXP3 GENE IS ASSOCIATED WITH A HIGHER INCIDENCE OF RELAPSE AFTER MYELOABLATIVE HLA-IDENTICAL SCT

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Introduction. The FOXP3 gene located on chromosome Xp11.23, encodes a protein that is a member of the forkhead/winged-helix family of transcriptional regulators. It is mainly expressed in CD4+CD25+ regulatory T cells, and is involved in the regulation of T-cell activation after Antigenic Stem Cell Transplantation. A microsatellite (GT)n polymorphism of the promoter/enhancer region of FOXP3 gene, located on the intron zero is associated with the degree of immunological reactivity mediated by this protein. Some studies suggest a lower reactivity when (GT)16 allele is present. However, the influence of the polymorphism in donor and recipient cells on the immunological phenomena developed after Allogenic Stem Cell Transplantation is unknown. Objective. The main objective of this study was to analyze the role of FOXP3/Scurfin gene intron zero (GT)n polymorphism in the outcome of myeloablative HLA-identical allo-SCT. Patients and Methods. Twenty-seven patients submitted to myeloablative HLA-identical allo-SCT at our institution were included in the analysis with a median follow up of 424 days (23-4369 days) were included in the analysis. Genomic DNA was purified from peripheral blood using a QIAamp DNA extraction kit (Qiagen). Genotyping of intron zero (GT)n polymorphism in the FOXP3 gene was performed by fluorescent PCR revealed by capillary electrophoresis as described by Bassuny et al. (Immunogenetics 55:149, 2003). Results. Distribution of (GT)n alleles in donors and recipients are summarized in Table 1. Since the FOXP3/Scurfin gene is located on the X chromosome, females can be homozygous or heterozygous while males are hemizygous. The presence of allele (GT)16 in the donor is associated with a higher relapse rate (50% vs 7.7%; p=0.085), and a lower Time to Progression (TTP; 426 days vs not reached, p=0.03). Patients transplanted from donors with allele (GT)16 showed less gr.III-IV acute and extensive chronic graft versus host disease incidence (p=NS; 6/11 vs 10/15). The higher incidence of relapse led to a worse event free survival (EFS) and overall survival (OS), although statistical significance was not achieved. Conclusions. The microsatellite (GT)n polymorphism in the promoter/enhancer region of the FOXP3 gene seems to influence outcome of myeloablative HLA-identical allo-SCT. Indeed, the presence of allele (GT)16 in the donor is associated with a higher incidence of relapse and a lower TTP. In the case these observations are confirmed in a larger patient cohort, they may be useful for an individualized management of transplanted patients.

OPTIMAL CUTOFF VALUE OF THE HEMATOPOIETIC PROGENITOR CELL (HPC) COUNT FOR EFFICIENT AUTOLOGOUS STEM CELL HARVEST

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Background. Even if enumeration of CD34+ cells in peripheral blood is a reliable index for timing efficient autologous stem cell collection (ASCC), Flow cytometric techniques to measure CD34+ cells are complex and costly. We previously have shown that hematopoietic progenitor cell (HPC) count enumerated by SE-9000 automated hematology analyzer is a useful surrogate for the timing of ASCC. Aims. We aimed to fine-tune cutoff value of HPC in predicting successful ASCC. Methods. Between May 2002 and January 2011, 578 patients (median age, 50 years; Male:Female = 230:148) with hematologic malignancy including 152 patients with multiple myeloma, 196 with Non-Hodgkin’s lymphoma, 23 with Hodgkin’s lymphoma and 7 with POEMS syndrome, underwent ASCC in the Asan medical center. A receiver operating characteristic (ROC) curve was used to define a threshold value of collected CD34+ cell count on day 1 and HPC for optimal ASCC. Results. We defined cutoff parameter for optimal CD34+ cell collection was a number of CD34+ cells collected on day 1 ≥ 2.0 x 10^6/kg (AUC 0.805, 95% confidence interval [CI] 0.759 - 0.848, p < 0.001) and the best cutoff value of HPC was 20 x 10^6/L (HPC 20) for the collected CD34+ cell count on day 1 ≥ 2.0 x 10^6/kg (sensitivity of 70.0% and specificity of 77.7%). On the basis of HPC 20 (HPC ≥ 20 x 10^6/kg, HPC <20), optimal collection rates were 94.8% and 75%, respectively. Failure to achieve optimal CD34+ cell collection was significantly associated with prior exposure to alkylating agents (p <0.001, odds ratio [OR] 2.86 [95% CI, 1.64 - 4.92] and the number of prior chemotherapeutic regimens (<2 vs. ≥2, p =0.004, OR 2.30 [95% CI, 1.30 - 4.06]) in the multiple logistic regression analysis. Summary/Conclusions. We defined cutoff value of HPC to be 20 x 10^6/L for optimal ASCC. Prior exposure to alkylating agents or heavy treatment was a significant cause for inefficient ASCC.

EFFICACY AND TOXICITY OF THE FLAMSA/RIC REGIMEN IN 40 PATIENTS WITH HIGH-RISK AML: A SINGLE CENTRE EXPERIENCE

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Background. Sequential use of chemotherapy and reduced-intensity conditioning (RIC) for allogeneic stem cell transplantation (SCT) in high-risk leukemia patients (pts) represents a promising approach (Schrijver et al., JCO 23, 2005: S675-S7). Here we present our experience with this therapy at cohort of 40 pts with acute myeloid leukemia (AML). Methods. High-risk was defined by progressive or refractory AML (n = 23), AML on the second or third remission (n = 8), or AML on the first remission with unfavorable cytogenetics (n = 9). Fludarabine (30 mg/m2), cytarabine (2 g/m2), and amsacrine (100 mg/m2) for 2 days followed. Prophylactic donor lymphocyte transclusions (pDLT) were given from day +120 in pts who were free of immunosuppressive medication for at least 30 days without developing GVHD. We analyzed 40 pts with AML undergoing FLAMSA/RIC in our centre from March 2006 to March 2010. Disease status before SCT was: CR1, n=9; CR2, n=6; CRs, n=1; PR1, n=1; refractory/refractory progressive disease, n=28. Median age of pts was 49 years (range 25-62). Types of donors and used grafts were as follows: HLA matched family (n=3), unrelated donor, n=30; PBSC, n=37; BM, n=3. Results. The median time of neutrophil engraftment (above 500/mm3) was 16 days, 34 pts engrafted, 6 pts died in aplasia on days 1, 3, 7, 8, 10 and 19 after SCT. Incidence of acute GVHD was...
evaluated in 33 pts: 58% (19/33) of pts had GVHD (grade I-II in 16 pts, grade III in 5 pts). Incidence of chronic GVHD was observed in 28 pts, 64% (18/28) of pts had GVHD (limited in 14 pts, extensive in 4 pts). Eight pts fulfilled the criteria for pDLT and received pDLT (median 2 doses). So far, 2 pts of 8 have got AML relapse and they died, 6 pts of 8 are alive in remission of AML.

The cumulative incidence of non-relapse mortality (NRM) at 1 year and 2 years was 28% (11/40) and 36% (14/39), respectively. Of deaths attributed to refractory cytopenias, posttransplant lymphoproliferative disease (1) and septic shock (n=4), multiorgan hemorrhage (n=3), brain hemorrhage (n=1) and posttransplant lymphoproliferative disease (1). The other most frequent toxicities were grade III/IV infections according to common toxicity criteria in 22 of 34 pts and gastrointestinal toxicity (grade III in 10 pts). Complete response to HSCT was achieved in 34 pts (97%), with a median follow-up from SCT of 33.5 months (range 10.60-10). The 1- and 2-year progression-free survival (PFS) was 70% (95% CI: 58-80) and 56% (95% CI: 42-69), respectively. Twenty-four pts died (12 deaths from NRM, 12 deaths from relapse or organ failure), 16 pts are alive and disease free. Conclusion. FLASMA/RIC regimen seems to be feasible and effective alternative for pts with high-risk AML with acceptable toxicity and high response rate (82%), 2-year OS after SCT is 41%.

**0988**

**ALLOGENEIC STEM CELL TRANSPLANTATION FOR ADULTS WITH MYELODYSPLASTIC SYNDROMES: RELEVANCE OF PRE-TRANSPLANT DISEASE STATUS**

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**Background.** Allogeneic haematopoietic stem cell transplantation (HSCT) is currently the only potential curative therapy for myelodysplastic syndromes (MDS). Approximately 40% of patients may be cured with HSCT, although advanced age, medical comorbidities and the lack of suitable donor limit this strategy to a selected minority of patients. Aim of the study was to investigate the outcome of adult patients with MDS who received an allogeneic HSCT in two main Piedmont Hematological Institutions.

**Methods.** We retrospectively analyzed 77 adult MDS patients (median age 53, 30-70 years) receiving an allogeneic HSCT between January 1995 and September 2010 at two main Piedmont Hematological Institutions. Patients were classified according to standard FAB criteria: 8 (10%) had refractory anemia, 10 (13%) refractory anemia with ringed sideroblasts, 46 (47%) refractory anemia with excess of blasts, 8 (10%) refractory anemia with excess of blasts in transformation, 14 (19%) chronic myelomonocytic leukemia and 8 (10%) had MDS not otherwise specified. At the time of diagnosis, 3 patients (4%) had IPSS low-risk disease, 19 (25%) had intermediate-1, 25 (33%) intermediate-2 and 9 (12%) intermediate-3 disease according to the revised IPSS criteria. After a median of 16 days after transplantation (range 11-30 days) and platelet engraftment was achieved in 71 patients (92%) at a median of 14 days (range 7-51 days). The cumulative incidence of acute graft-versus-host disease (aGVHD) by day +100 and chronic GVHD by 1 year were 30% (19-41%), 52% confidence interval (CI) and 35% (45-68%, 95% CI) respectively. The cumulative incidence of transplant-related mortality (TRM) at 100 days and 1 year were 13% (5%-21%, 95% CI) and 20% (11%-29%, 95% CI) respectively. The 2-year progression-free survival (PFS) and overall survival (OS) were 41% (95% CI: 24-58) and 53% (95% CI: 36-69) respectively. On univariate analysis, advanced disease status at transplantation was the major independent variable associated with an inferior 2-year PFS (HR 4.48, 95% CI, p<0.001) and OS (HR 4.11, 1.94-8.70 95% CI). The use of a donor other than an HLA-identical sibling (HR 2.31, 1.10-4.73, 95% CI, p=0.04) was the independent variable associated with TRM. Summary/Conclusions. Our data suggest that disease status at the time of transplant is the major predictor for improved PFS and OS, and treatments required to reach this goal may have value in leading to improved outcome. Additional studies are justified for clarifying the role of HSCT in MDS.

**0989**

**CYTOMEGALOVIRUS INFECTION IN PATIENTS WITH LYMPHOMA UNDERGOING AUTOLOGOUS TRANSPLANTATION: A RETROSPECTIVE ANALYSIS FROM THE ROME TRANSPLANT NETWORK**

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**Background.** Routine monitoring for Cytomegalovirus (CMV) infection are considered unnecessary in patients undergoing autologous hematopoietic stem cell transplantation (ASCT) because of the low likelihood of progression from infection to disease, with the exception of high-risk subgroups, including those receiving CD34-selected grafts and prior treatment with Fluadribine, Cladribine or Alentuzumab. However, current data on CMV infection and disease following ASCT for malignant lymphoma are very limited. The starting point for this study was the quality accreditation process of the Rome Transplant Network (RTN) according to the JACIE standards, considering that two different CMV infection diagnostic strategies were set within the participating institutions: a clinically driven diagnostic approach based on symptoms and/or organ involvement and a PCR monitoring strategy in patients with clinical signs suggesting a CMV infection and a CMV surveillance based on a routine monitoring of all patients by plasma quantitative PCR assay. Aims. The aim of the study was to compare two different CMV infection diagnostic strategies (i.e. clinically driven vs surveillance) in terms of CMV symptomatic infection and/or end-organ disease incidence, diagnostic timing, transplant-related mortality (TRM), CMV-related mortality (CMVRM), PCR testing cost and to provide insights on the risk factors for CMV symptomatic infection or end-organ disease and TRM. CMV symptomatic infection and end-organ disease were published in previous investigations. Methods. We perform a retrospective analysis on 144 adult patients (median age 47 years, range 17-71) with diagnosis of malignant lymphoma consecutively undergoing non-selected peripheral blood ASCT in 3 Hematology Institutions participating in the RTN. Results. Considering the two CMV infection diagnostic strategies, PCR testing cost was significantly higher in the surveillance arm ($199 vs $264, p<0.001), whereas no statistically significant difference was observed concerning CMV symptomatic infection or end-organ disease incidence (11.5 vs 10.5%, p=1), diagnostic timing (day 33 vs 26, p=0.294), TRM (7 vs 3.5%, p=0.422) and CMVRM (4.9 vs 0%, p=0.116). In multivariate analysis, the HBcIgG seropositivity [HBcIgG- HR 0.13 (95% CI: 0.03-0.6), p=0.004] and conditioning regimens containing 90Y-Ibritumomab Tiuxetan (Z-BEAM or Z-FEAM) [HR 28.5 (95% CI: 2.9-278.6), p=0.004] or non-Carmustine-based [HR 34.8 (95% CI: 5.2-231.9), p=0.000] were independent variables associated with TRM. Summary/Conclusions. Routine monitoring for CMV infection following ASCT should be carried out not only in lymphoma patients grafted with CD34-selected grafts but also in patients treated with Fluadribine, but not in lymphoma patients HLA-ab+. For our study, 90Y-Ibritumomab Tiuxetan in conditioning and non-BCNU-based regimens should be considered as further risk factors for CMV symptomatic infection or organ disease.
0990 INTRABONE CORD BLOOD TRANSPLANT: PRELIMINARY RESULTS FROM A PROSPECTIVE PHASE II STUDY

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Summary/Conclusions. Preliminary results of this study with advanced disease suggest that intrabone injection of CB resulted in short term good engraftment, especially for platelets, low GVHD and good outcome. Longer follow up is needed to estimate the actual antileukemic effect.

0991 UNRELATED STEM CELL TRANSPLANTATION IN ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA: HLA-MATCHING DEGREE AND GRAFT SOURCE-BASED ANALYSES OF LONG-TERM OUTCOMES

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Background. Adults with high-risk acute lymphoblastic leukemia (HR-ALL) have a poor outcome with standard chemotherapy and usually undergo unrelated donor stem cell transplantation (URD-SCT) if a matched sibling donor is not available. However, long-term results of URD-SCT, especially based on HLA-matching degree and graft source, are scarce in adult HR-ALL patients. Aims. We report long-term outcomes in 106 consecutive adult HR-ALL patients who received URD-SCT using bone marrow (BM; n=67) or peripheral blood progenitor cells (PBPC; n=39) at our center between 2000 and 2008. Methods. Median age was 24 years (range, 15-54 years). All patients had one or more high-risk features, including adverse cytogenetics [t(9;22), t(15;17), t(8;14), complex, Ho-Tr], older (P=0.050) and received a reduced-intensity conditioning regimen (P=0.015). Disease status at the time of transplantation was more advanced for 7/8-matched BM transplants (P=0.009). After a median follow-up of 53 months (range, 24-124 months) for surviving transplants, the 5-year cumulative incidence of relapse and non-relapse mortality were 31% and 25%, respectively, and the 5-year disease-free survival (DFS) rate was 25%. The risk of relapse was higher for 7/8-matched transplants (42%; 48% for PBPC and 37% for BM) than for 8/8-matched transplants (19%; 18% for PBPC and 20% for BM; P=0.019), while the risk of non-relapse mortality was similar between groups. As a result, DFS at 5 years was lower using 7/8-matched grafts (44%; 42% for PBPC and 46% for BM) than 8/8-matched grafts (62%; 59% for PBPC and 65% for BM; P=0.043). In each group of patients showing the same HLA-matching degree, overall transplantation outcomes were similar between PBPC and BM transplants. Regardless of HLA-matching degree and graft sources, disease status at transplantation (CR1 versus beyond CR1) was an independent predictive factor affecting relapse (HR 2.51, 95% CI 1.64-4.57; P=0.004) and DFS (HR 2.75, 95% CI 1.41-5.36; P=0.003) in univariate analysis. The presence of chronic GVHD was also associated with lower relapse (HR 5.06, 95% CI 2.01-12.61; P=0.001) and better DFS (HR 2.37, 95% CI 1.23-4.57; P=0.010). Summary/Conclusions. Our long-term data suggest that outcomes are similar for transplantation using PBPC or BM sources in the setting of 8/8-matched or 7/8-matched URD-SCT for adult HR-ALL.
lapse was 25% and at one year, PFS was 44%. At day +100, NRM was 15% and overall NRM was 25% (2 patients due to acute GVHD and 3 infectious complications). Median OS has not been reached. 2-year estimated OS was 54%. Conclusions. Our results show that yttrium-90-ibritumomab tuxetan as a component of reduced intensity conditioning for allogeneic transplantation is feasible in patients with high-risk relapsed or refractory aggressive B-cell lymphoma. Longer follow up is needed in order to design future trials.

0993

CLINICAL IMPACT OF GLUTATHIONE S-TRANSFERASE M1 POLYMORPHISM ON OUTCOME AFTER ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Despite significant progress in allogeneic hematopoietic stem cell transplantation (allo-HSCT), this procedure is still associated with substantial morbidity and mortality. Various pretransplantation and transplantation-related clinical risk factors have been implicated, but so far there is no method to estimate the occurrence and severity of transplant-related complications. The most common complication after allo-HSCT which affects survival remains graft versus host disease (GVHD). Single nucleotide polymorphisms in genes coding cytokines and chemokines have been shown to influence GVHD and outcome after allo-HSCT. Besides pharmacogenomics is a new field investigated in the HSCT setting. This study aimed to determine association between polymorphism of glutathione S-transferase M1 (GSTM1) genotypes with the outcome of allo-HSCT performed between May 2001 and October 2010 in our center. Materials and Methods. The allelic variants of GSTM1 gene were determined in 83 patient/donor pairs by real-time polymerase chain reaction. The most common complication after allo-HSCT is GvHD. Components of aGVHD: C282Y,n=15, het C282Y,n=7, het S65C,n=2, and homozygosity for H63D,n=3. Median SF was 73 (81%). Preparative regimen was conventional conditioning with 12 Gray TBI/cyclophosphamide 120 mg/kg in 32 (35.6%) and reduced in 73 (81%). For survival analysis, the patients were divided into three groups: 1) those who became PET positive post-transplant c. 2 relapses were recorded among 16 patients who were PET positive both pre- and post-ASCT, 2) those who became PET negative prior to ASCT but had a PET uptake at any time post-transplant disclosing: a. One relapse was recorded for those who were PET negative pre- and post-ASCT, vs 17 among 35 MRUp or positive ones, leading to a 2-year FFS of 77% vs 45%, respectively (p=0.02). Post ASCT PET scan had a strong prognostic impact on outcome: 2-year FFS was 87% for PET negative or MRUp patients vs 65% for positive ones, (p<0.0001). The analysis of the 50 patients who had a PET scan available both pre- and post-transplant disclosed: a. One relapse was recorded for those who were PET scan either positive or MRUp pre-ASCT and became negative or MRUp after ASCT (1/16 patients), b. 15 patients relapsed among 16, who were PET scan positive or MRUp pre-transplant and remained or became positive post-transplant c. 2 relapses were recorded among 16 patients who were negative both pre- and post-ASCT. These differences were statistically significant (p<0.0001). Conclusions. PET scan positivity prior to ASCT does not preclude a favorable outcome in patients with primary refractory/refractory HL, since approximately 50% remain disease free after ASCT. Patients who remain or become PET positive after ASCT have a dismal outcome in contrast to those who become PET negative.

0999

EXTRA- AND INTRA-CELLULAR IRON DISTRIBUTION IN PRE-TRANSPLANT BONE MARROW TREPHINE BIOPSIES AND OUTCOME AFTER ALLOGENIC HEMATOPOIETIC CELL TRANSPLANTATION

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Limited data are available on the patterns of pre-transplant iron deposition in the bone marrow (BM) [intracellular and/or in macrophages] and their impact on outcome [survival, non-relapse mortality (NRM), and graft-versus-host-disease (GVHD)] after allogeneic hematopoietic cell transplantation (HCT). Also, the correlation of BM iron stores (Fe-S) with inherited (HFE-genotype), treatment-related factors [pre-transplant units of packed red blood cells (URB) transfused] and serum ferritin (SF) is not clearly defined. Therefore BM-biopsies of 90 patients (AML, n=68, MDS, n=22), taken for diagnostic purpose at a median of 24 days pre-HCT, were analysed for iron deposition (Perl’s reaction) and macrophage identification (CD68 immunostaining). Grading (G0-G5) for Fe-S, and (G0-G3) for intracellular iron (Fe-I) was used to express iron deposition. The percentage of iron storing macrophages in relation to all CD68+ macrophages was measured 7 days pre-HCT with a median C-reactive protein of <5 mg/l [48 males/42 females, median PET Gamma camera]. Donors were matched-related (MRD) in 17 (19%) and matched-unrelated (MUD) in 73 (81%). Preparative regimen was conventional conditioning with 12 Gray TBI/cyclophosphamide 120 mg/kg in 32 (35.6%) and reduced intensity conditioning with fludarabine 30mg/m2/day for 3 days and 2 Gray TBI 15-55 (64.4%). Mutated HFE-genotype [C282Y,G631A] were present in 27 patients (28%). Using PET scan pre-HCT (heterozygosity) the rate for HFE631A was 15% and overall NRM was 25% (2 patients due to acute GVHD and 3 infectious complications). Median OS has not been reached. 2-year estimated OS was 54%. Our results show that yttrium-90-ibritumomab tuxetan as a component of reduced intensity conditioning for allogeneic transplantation is feasible in patients with high-risk relapsed or refractory aggressive B-cell lymphoma. Longer follow up is needed in order to design future trials.
Fe-S and both Fe-I and Fe-Mac (r=0.8, p<0.0001) and between Fe-I and Fe-Mac (r=0.8, p<0.0001) were strongly significant but augmented Fe-S was not associated with increasing Mac (p=0.4). Also, higher SF values were associated with higher grades for Fe-S (p=0.002), Fe-I (p=0.01), and Fe-Mac (p=0.004) but the correlation was poor (r=0.3).

There was a significant correlation of SF level (r=0.6, p<0.0001) but not of Fe-S and URB transfection. Additionally, Fe-S was not influenced by HFE-genotype. After a median follow-up of 24 months, acute and chronic graft-versus-host-disease (GVHD) occurred in 70% and 53% of patients respectively. Survival was 58% and NRM was 22%. Fe-S, Fe-I, and Fe-Mac did not correlate with GVHD, survival or NRM. However, elevated SF levels were highly predictive for inferior survival (p=0.002) and higher NRM (p=0.007) as in patients with SF >500 ng/mL, survival and NRM were 37% and 49% versus 78% and 9% respectively in patients with lower SF (p=0.001). In addition, SF levels >500 ng/mL were associated with acute GVHD (p=0.002) but not with chronic GVHD. In the face of transfusional iron overload, iron is deposited in the bone marrow equally both interstitially and in macrophages. Iron overload measured by an elevated SF is usually accompanied by augmented marrow iron stores but the correlation remains poor. The pre-transplant marrow iron status, unlike serum ferritin, could not predict outcome after HCT. Keeping the well-known limitations in mind, serum ferritin remains a cheap and non-invasive tool to measure iron overload.

Follow up was 17.24 months (0-61). Survival in the entire group was 75.9% (142 patients). MRT was 5.8% and MRT-100 3.2%, mainly due to infection and acute GVHD. Mean (SD) pre-HSCT serum ferritin concentration was 951 (1125) ng/mL in the entire group, 704 (929) ng/mL in autologous recipients, and 1357 (1291) ng/mL in allogeneic recipients. Ferritin level was <500 ng/mL in 82 patients (43.9%), 500-1000 in 46 patients (24.6%), and >1000 in 59 patients (31.6%). High ferritin concentrations (500-1000 ng/mL and >1000 ng/mL) were significantly associated with overall survival (79.3%, 65.1% and 37.2% respectively; log rank test p=0.044) (figure 1). Ferritin level >600 ng/mL was significantly associated with higher mortality secondary to infection (89% vs 72.4%, p=0.035). We didn’t found association between serum ferritin level and MRT, MRT-100 or aGVHD (grade 0-2 vs 3-4). Summary/Conclusions. In our serie pre-transplantation serum ferritin level divides patients into 3 groups of risk with significantly different survival. Ferritin level was also associated with higher risk of mortality secondary to infection. This study suggest a predictive role of pre-transplantation ferritin levels in selecting a subset of patients at increased risk for HSC, and could be useful in making treatment decisions for individual patients.

**0997**

**THE IMMUNE PHENOTYPIC PROFILE OF HEMATOPOIETIC STEM CELLS IN DIFFERENT MOBILIZATION REGIMENS AND GENE EXPRESSION PROFILES OF G-CSF MOBILIZED PERIPHERAL HEMATOPOIETIC STEM CELLS**

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**Background.** There is no detailed information about immune phenotypic profile of hematopoietic stem cells (HSC) in different mobilization regimens and gene expression profiles of G-CSF mobilized HSC products. The effects of these properties on the outcome of transplantation largely unknown. Aims and Methods. In this prospective study, 114 peripheral immune phenotypic features (PE-CD11a, FITC-CD18, PE-CD31, APC-CD38, FITC-CD44, APC-CD62e, FITC-CD62L, FITC-CD90, PE-CD117, PE-CD135, PE-CD184) of HSCs which had been mobilized with three different regimens (group I: growth factor alone, group II: cyclophosphamide + growth factor, group III: ESHAP + growth factor) from a total of 44 patients (median age: 46 F/M: 18/28) have been investigated. CD34 + cell sorting was done by flow cytometry (BD FACSaria cell sorter). The relationship between HSC immune phenotype and the duration of the neutrophil and platelet engraftment has also been studied. Additionally, in 9 stem cell products (without cell sorting) which had been mobilized by using G-CSF alone, whole genomic expression profiling was carried out. Moreover the effects of these gen profiles on graft versus host disease (GVHD) was evaluated. Results. The median duration of neutrophil engraftment after transplantation was 12 (9 - 21) days and platelet engraftment was 12 (7 100) days. The immune phenotypic features of group I, II and III mobilized HSs were not significantly different. The surface antigens most commonly expressed by CD34 positive stem cells were CD31, CD44, CD90, CD117 and CD185. The CD31 (platelet endothelial cell adhesion molecule-1) positivity ratio of the HSCs were inversely correlated with the duration of the neutrophil (r= -0.32, p= 0.05) and platelet (r= -0.36, p= 0.02) engraftment. No relationship was found between engraftment duration and the expression status of CD184 (CXCR4). There was a relationship between the acute GVHD and the increased expression of genes that are associated with immunity, cell communication and metabolic processes. Another relationship was also found between the development of chronic GVHD and the expression levels of genes which are related to growth and metabolic processes. Conclusions. As a result we found that the surface immune phenotypic profiles of CD34+ peripheral HSCs harvested by different mobilization regimens were not different. To our knowledge, it has been demonstrated for the first time that CD31 expression of HSC could positively affect both neutrophil and platelet engraftment. Additionally, CD184 (CXCR4) expression ratio and even CD184 negativity of HSCs have found no effect on neutrophil or platelet engraftment. When these results are evaluated, additional surface antigens (such as CD31) might be more effective in the homing process. More studies investigating the effects of stem cell products whole gen expression on graft versus host disease are needed.

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IS THERE STILL A ROLE FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA?

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Background. the role of autologous stem cell transplantation (ASCT) in the treatment of patients with acute myeloblastic leukemia (AML) remains unsettled. Aims. we report our results of ASCT in patients with AML during the last 15 years. Methods. between December 19, 1994 and May 10, 2010, a total of 63 patients with AML without HLA-matched donor in the department of Hematology and Transfusion Medicine, University Hospital, Bratislava, received an ASCT. The median patient age was 41 years (range 20-61 years). 35 (56%) males and 28 (44%) females. At the time of ASCT, 50 (79%) patients were in first complete remission (CR), 11 (18%) patients were in second CR and 2(3%) patients were in relapse. The median time interval from CR to ASCT was 107 days (range 48-281). Five (8%) patients received bone marrow (BM), 53 (84%) patients received peripheral blood stem cells (PBSC) and 5 patients (8%) received BM plus PBSC. Patients were stratified into three risk groups; poor, intermediate and good-risk on the basis of cyto genetic and molecular analyses at diagnosis. Results. with a median follow-up of 92 months (7.6 years), the 10 year overall survival (OS) and disease free survival (DFS) of all patients is 55% and 51%, respectively (Figure 1). Transplant-related mortality is 6%. The relapse rate is 35% and 9 years probability of relapse is 44%. Low white blood cell count (WBC) at diagnosis, favorable and intermediate cytogenetic-risk was independently associated with clinical outcome by univariate analysis. Conclusion. we conclude that ASCT is still an effective post-remission treatment in AML patients without HLA-matched donor, with the possibility of long-term survival or even cure in remarkable proportion of patients with AML, particularly in patients with favorable and intermediate cytogenetic risk.

OUTCOMES OF REDUCED-INTENSITY STEM CELL TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA WITH T(8;21) IN COMPARISON WITH CONVENTIONAL STEM CELL TRANSPLANTATION

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Background. Patients with t(8;21) generally have a favorable prognosis as candidates for stem cell transplantation (SCT). However, some studies raised questions regarding improved survival with autologous SCT (ASCT) in these patients. Moreover, there has been some concern that the rate of relapse after HDARA-C is higher than previously reported and although approximately 50% of CBF-AML patients achieve long-term survival, disease relapse is still a major cause of treatment failure. Of CBF-AML, t(8;21) patients have a risk score of 2-3 (53.7%; n=67). None of the patients with a score of 0-1 (n=18) had a significantly improved 100-day survival (100%) compared to patients who had a risk score of 2-3 (53.7%; n=67). Patients with a score of 0 had 100-day survival of 100%. We propose that based on this risk score, patients with a positive fungal isolate and a score of 0-1 do not require treatment whereas patients with a score of 2-4 are at increased risk of mortality and should be considered for anti-fungal therapy. A prospective study to validate the fungal isolate score is underway.

DEVELOPMENT OF RISK SCORE TO PREDICT SIGNIFICANCE OF POSITIVE FUNGAL ISOLATES IN RECIPIENTS OF ALLOGENEIC AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Background. Invasive fungal infections are a major cause of mortality following stem cell transplantation (SCT). The significance of a positive microbiological isolate with yeasts and molds from any site in SCT recipients. Methods. we retrospectively reviewed all microbiological data, including cultures and direct microscopic examination for yeasts and mold species in 760 autologous SCT (auto-SCT) and 455 allogeneic SCT (allo-SCT) recipients at our institution between 1997 and 2006. Specific fungal species, predisposing factors and survival rates at 100 days after a positive fungal isolate were analysed. Results. a total of 494 positive fungal isolates were documented in 187 patients. Fungal isolates were reported in 100 (22%) allo-SCT and 87 (11.4%) auto-SCT recipients. Of these, 407 were yeasts and 87 filamentous fungi. Non-albicans Candida accounted for 57% of all yeasts and Aspergillus species for 74% of filamentous fungi. The 100-day overall survival rate was 56% for auto-SCT and 59.4% for allo-SCT recipients (p=0.72). In allo-SCT recipients with a positive fungal isolate, the following risk factors were associated with significantly lower 100-day survival on univariate analysis: Neutropenia (57% vs. 51%, p=0.024), immunosuppressive treatment (29% vs. 7%, p=0.053), fungemia (57.7% vs. 65%, p=0.018), isolation of non-albicans Candida and aspergillus species (aspergillus species 57.4% vs. non-albicans Candida 46.8% vs. C. Albicans and other yeasts 75%, p=0.021), in vitro resistance to >1 antifungal drug (none=65% vs. one=50% vs. two=53.3%, p=0.009), lymphopenia <1x10^9/L (41% vs. 56%, p=0.005), concurrent fever for >24 hours (52% vs. 66%, p=0.008), respiratory symptoms (20% vs. 78%, p=0.001) and presence of a central line (48% vs. 53%, p=0.004). In auto-SCT recipients with a positive fungal isolate, the only factors associated with lower 100-day survival were creatinine >120 µmol/L (p=0.0004) and fungemia (p=0.005). Previous history of organ transplantaion, multifungal isolates, abnormal chest X-ray or CT scan were not found to be associated with lower survival. In multivariate analysis only three factors, namely lymphopenia, creatinine >120 µmol/L and respiratory symptoms at the time of isolation remained significant for day-100 survival. Based on these results, we developed a fungal isolate risk score for allo-SCT recipients where each adverse score was assigned two scores, the presence of respiratory symptoms (yes=2 or no=0), creatinine >120 µmol/L (yes=1 or no=0) and lymphopenia (yes=1 or no=0). Patients with a score of 0-1 (n=18) had a significantly improved 100-day survival (100%) compared to patients who had a risk score of 2-3 (53.7%; n=67). None of the patients with a score of 4 (n=4) survived at 100 days. We propose that based on this risk score, patients with a positive fungal isolate and a score of 0-1 do not require treatment whereas patients with a score of 2-4 are at increased risk of mortality and should be considered for anti-fungal therapy. A prospective study to validate the fungal isolate score is underway.
two FBA regimens among adult patients transplanted in this monocenter series it seems that a higher dose freezing temperature does not affect seriously the PBPCs quality. However, viability, CD34+ cells, CFU-GM and bacterial contamination are comparable in both groups.

Summary/Conclusions. Results. A total of 123 patients, with 268 PBPCs LAPH (92 in group A and 176 in group B), were included. The median, aged was 49 years (3-73). Diagnoses were: MM, 45, breast cancer, 22 NHL, 12 HD and 26 others diagnoses. Mobilization was done: with chemotherapy+G-CSF (15.1%), cyclophosphamide+G-CSF (21.8%) or G-CSF (26%). Half of the LAPH were IF and half were ON stored: 46 LAPH at 4ºC, and 88 at 20ºC. In the group A there were 92 LAPH (46 IF and 46 ON), and in the group B there were 176 (88 IF and 88 ON). In group A no significant difference was found between the IF CD34+ recovery (127.7±67.4) compared with those ON stored at 4ºC (112.1±43.2). There were not significant differences in group B between IF (121.5±91.7) and 20ºC (118.7±123.6). When the recovery of CFU-GM in group A, IF and ON storage, were compared no difference was found; the same occurred in group B. However, the pre-freezing viability was significantly decreased in the ON samples in both groups, in comparison with IF (86.7±56.2% vs 92.5±7.5% in group A) and (76.6±15.7% vs 86.0±11.7% in group B). No statistical difference was found when viability, CFU-GM and CD34+ cells recovery between both groups were compared. The bacterial contamination incidence was not different in both groups. Conclusions. These data suggest that the ON pre-freezing temperature does not affect seriously the PBPCs quality. However, viability decreases when freezing is not performed immediately.


test was used to compare CD34+ cells, CFU-GM and viability, pre-freezing and post-thawing, and the recovery with both temperatures. Chi-square-test was used for bacterial contamination comparison. Results. A total of 123 patients, with 268 PBPCs LAPH (92 in group A and 176 in group B), were included. The median, aged was 49 years (3-73). Diagnoses were: MM, 45, breast cancer, 22 NHL, 12 HD and 26 others diagnoses. Mobilization was done: with chemotherapy+G-CSF (15.1%), cyclophosphamide+G-CSF (21.8%) or G-CSF (26%). Half of the LAPH were IF and half were ON stored: 46 LAPH at 4ºC, and 88 at 20ºC. In the group A there were 92 LAPH (46 IF and 46 ON), and in the group B there were 176 (88 IF and 88 ON). In group A no significant difference was found between the IF CD34+ recovery (127.7±67.4) compared with those ON stored at 4ºC (112.1±43.2). There were not significant differences in group B between IF (121.5±91.7) and 20ºC (118.7±123.6). When the recovery of CFU-GM in group A, IF and ON storage, were compared no difference was found; the same occurred in group B. However, the pre-freezing viability was significantly decreased in the ON samples in both groups, in comparison with IF (86.7±56.2% vs 92.5±7.5% in group A) and (76.6±15.7% vs 86.0±11.7% in group B). No statistical difference was found when viability, CFU-GM and CD34+ cells recovery between both groups were compared. The bacterial contamination incidence was not different in both groups. Conclusions. These data suggest that the ON pre-freezing temperature does not affect seriously the PBPCs quality. However, viability decreases when freezing is not performed immediately.


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impact hematopoietic stem cell (HSC) mobilization, including baseline platelet counts. Thrombocytopenia prior to mobilization with G-CSF±chemotherapy is a significant predictive factor for mobilization failure in patients with Hodgkin’s and non-Hodgkin’s lymphoma (Hosing C, *Am J Hematol.* 2009). *Aims.* The purpose of this retrospective analysis is to assess the efficacy of HSC mobilization with plerixafor plus G-CSF in patients with low platelet counts. *Methods.* Patients who had failed at least 1 previous HSC mobilization were remobilized with plerixafor plus G-CSF as part of the European compassionate use program (CUP). G-CSF (10 µg/kg) was administered subcutaneously (SC) every morning for 4 days and plerixafor (0.24 mg/kg SC) was administered in the evening on Day 4. On Day 5, G-CSF was administered and apheresis was initiated. Plerixafor, G-CSF and apheresis were repeated daily until patients collected the minimum of 2x10^6 CD34+ cells/kg. *Results.* Data on platelet counts prior to mobilization are available for 189 patients from our European CUP database. Of these, 86 patients presented platelet counts ≤150x10^3/L, median 115, range 18-150; NHL=40, MM=32, HL=10, other=4; 55% male; median age 57 years, range 20-75; median number of prior therapy regimens 3, range 1-10, and 103 patients had normal platelet counts >150x10^3/L, median 225, range 151-442; NHL=45, MM=37, HL=21; 58% male; median age 54 years, range 24-72; median number of prior therapy regimens 2, range 1-4. Following a similar number of aphereses in both cohorts (median 2, range 1-4), the median CD34+ cell yield was significantly higher in the normal platelet group (3.2x10^6/kg, range 0.5-32.6) than in thrombocytopenic patients (2.56x10^6/kg, range 0.18-9.2; p<0.001). Also a significantly higher proportion of patients achieved the target cell dose of 2x10^6 CD34+ cells/kg in the normal platelet group (86% versus 58%, p<0.001). Sixty-five per cent of patients with normal platelet counts and 45% in the thrombocytopenic group have undergone ASCT, with similar time to neutrophil and platelet engraftment in both groups. *Conclusions.* In keeping with previous reports in first line mobilization, our data suggest that thrombocytopenia prior to mobilization with plerixafor and G-CSF remains a significant predictive factor for mobilization failure compared with patients with normal platelet counts. Nevertheless, 58% of such thrombocytopenic patients, who have already failed prior mobilization attempts, can be successfully rescued with plerixafor and G-CSF to collect ≥2x10^6 CD34+ cells/kg. Overall, this strategy provides a remarkable success rate in the mobilization of these challenging patients and subsequently proceeding to transplantation, compared with published alternatives for thrombocytopenic patients.

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**VARIABLES AFFECTING HEMATOPOIETIC STEM CELL MOBILIZATION FOR HIGH DOSE CHEMOTHERAPY AND STEM CELL RESCUE IN CHILDREN**

J Sevilla, M Guillén, A Castillo, C Hernandez, M Cormenzana, MA Andion, M Gonzalez-Vicent, A Lasaletta, A Perez, L Madero, M Diaz

**Background.** A SDF-1alpha/CXCR4 binding inhibitor has been recently approved for adult patients diagnosed with multiple myeloma or lymphoma to enhance mobilization of hematopoietic stem cells (HSC) to peripheral blood (PB). Experience in children, however, is extremely limited. We have conducted a retrospective study in order to analyze variables related to poor mobilization in children to identify those patients who will likely benefit on the use of this new drug. *Aims.* To analyze variables related to poor mobilization of peripheral blood progenitor cells in children. *Methods.* We analyzed data prospectively recorded, since January 2000, from 183 consecutive patients mobilized with filgrastim at different doses prior to HSC collection. CD34+ cells on PB before the first apheresis were evaluated to consider poor mobilizers. Patients with less or equal to 10 CD34+ cells/mcl on PB were considered poor mobilizers independently if they underwent collection or not. In our experience most children reach the target CD34+ cell dose after several procedures, even those considered as poor mobilizers, but in this study we tried to identify those who will likely benefit of new mobilization agents. All procedures were performed in the Transfusion Service at Hospital Infantil Universitario Niño Jesús (Madrid). Patients’ diagnosis were as follows: central nervous system tumours 51(28%), Ewing sarcoma 42 (23%), neuroblastoma 36 (19%), rhabdomyosarcoma 3 (2%), Wilms tumour 3 (2%), Osteosarcoma 6 (3%), Non-Hodgkin lymphoma 20 (11%), Hodgkin disease 12 (6%) and others 9 (5%). Disease status was: first complete remission 107 (59.1%), partial remission 44 (24.3%), disease progression 13 (7.7%), and complete remission after first relapse 17 (9.4%). Median age was 7 years (1-18), and median weight 25 kg (5-111). Mobilization regimens included: G-CSF 12mcgr/kg/12h (64.9%), 10mcgr/kg/24h (3.51%), 12mcgr/kg/24h (24.31%), 10mcgr/kg/12h (7.18%), others (1.1%). Contingency table was applied for categorical variables on the univariate analysis. Correlations were determined using logistic regression. *Results.* Median CD34+cells on PB before the collection was 40 (1-495). Twenty-six patients had less or equal than 10 CD34+ cells/mcl before the collection (14%). Of those, only 7 patients did not undergo HSC collection. Several variables were studied, but only radiotherapy was related on univariate analysis to poor mobilization (p=0.01). Other variables showed clear tendency but did not reach statistical significance: type of mobilization (p=0.14); age (p=0.15); diagnosis (p=0.06); disease status (p=0.08). We define a risk score giving one point each to radiotherapy, chemotherapy more than 6 cycles of chemotherapy before the mobilization, and age older than 9 year old. Those patients with 2 or 3 points have higher risk of mobilization failure (p=0.01). When these variables were included in a multivariate analysis, only prior radiotherapy HR 0.08 (0.18-0.35) p = 0.0009; and mobilization with 12 mcgr/kg/day HR 0.29 (0.09-0.95) p=0.04, were related to poor mobilization. *Conclusion.* Prior use of radiotherapy is the main variable related to poor mobilization in children. No single variable is helpful to anticipate the need for using new mobilization agents in children. Only those patients with several risk factors may be candidates to be considered as potentially at risk for poor mobilization.
SIMULTANEOUS SESSION II

Therapy for relapsed/refractory Multiple Myeloma

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PHASE 2 RANDOMISED OPEN LABEL STUDY OF 2 MODALITIES OF POMALIDOMIDE (CC4047) PLUS LOW-DOSE DEXAMETHASONE IN PATIENTS WITH MULTIPLE MYELOMA, REFRACTORY TO BOTH LENALIDOMIDE AND BORTezOMIB. IFM 2009-02

X Leleu,1 M Attal,1 B Arnulf,1 A Duhamel,1 P Moreau,1 C Trauille,4 G Marit,1 M Michalet,1 C Mathiot,1 M Pettillon,1 M Macro,11 M Rousell,1 C Hulin,15 B Pégourié,1 B Kolb,1 AM Stoppa,16 S Brenichia,17 L Garderet,18 B Royer,1 L Benboubker,19 D Caillot,1 O Decaux,2 M Escoffre-Barbe,2 J L Harousseau,16 H Avet-Loiseau,1 T Facon1

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Background. Patients with multiple myeloma (MM) who are refractory to bortezomib and lenalidomide (double refractory) have limited treatment options and less than a year of life expectancy. In prior phase III studies of pomalidomide in double refractory patients, limited activity was observed, open-label study aimed to determine the efficacy and toxicity profile of 2 modalities of pomalidomide in double refractory patients.

Method. The patients had symptomatic, progressive, measurable and double refractory MM. The primary objective was to determine response rate (RR) and event-free survival (EFS) to pomalidomide and dexamethasone using IMWG response criteria. The response and FISH cytogenetic analysis were assessed centrally. Pomalidomide was given orally either 4 mg daily on days 1-21 of each 28-days (arm A) or continuously on days 1-28 of each 28-days (arm B). Response was evaluated using the Bladé criteria. Treatment: Patients were randomized to either Arm A (bortezomib 1.3 mg/m2 on days 1, 4, 8, 11 every 21 days) or Arm B (bortezomib 1.3 mg/m2 on days 1, 4, 8, 11 every 21 days). Patients were evaluable for response evaluation in arm A and B, respectively. In arm A, 12 (30%) patients had PR and better, including 3 VGPR, and 21 (52%) had stable disease. In arm B, 17 (47%) patients had PR and better, including 1 VGPR, and 15 (41%) had stable disease. The median duration of response was 77 and 89 days in arm A and B, respectively. Twenty three (57%) and 22 (61%) patients remained progression free, and 5 patients have died in either arm, respectively. Toxicity (at least possibly related to treatment) consisted primarily of myelosuppression in both arms. The occurrence of neuropathy was not observed nor worsening of pre-existing neuropathy. No thromboembolic events have occurred. Conclusion. Pomalidomide and dexamethasone is active and well tolerated in this heavily pre-treated population of lenalidomide and bortezomib-refractory MM patients. This study provides further evidence that pomalidomide has no-cross resistance with lenalidomide and suggests that it can provide benefit for patients who have relapsed after other novel therapies. Final results will be provided at EHA 2011.

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RESULTS OF AN INTERNATIONAL, RANDOMIZED PHASE II CLINICAL TRIAL OF BORTezOMIB (TRAIL-R1 AGONIST MONOCLONAL ANTIBODY) FOR THE TREATMENT OF RELAPSED/REFRACTORY MULTIPLE MYELOMA (MM)

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8 Roswell Park Cancer Institute, Buffalo, United States of America
9 London, United Kingdom, June 9 – 12, 2011

Background. Development of new therapies for patients with relapsed/refractory MM remains an important clinical need. Monoclonal antibodies (mAb) have made an important impact in B-cell cancers especially when combined with chemotherapeutics. Unfortunately, currently there are no approved mAb for MM patients. Mapatumumab is a fully human mAb that targets and activates the TRAIL-R1 receptor. In preclinical MM models, enhanced efficacy of bortezomib was noted when combined with mapatumumab. Aims. Based on these preclinical observations, a randomized phase II clinical trial was designed to evaluate the clinical efficacy of mapatumumab when added to bortezomib. Methods. Informed consent was obtained from all patients participating in this study. Patients with relapsed or refractory MM, who had measurable M-protein in serum and/or urine and had failed up to 2 prior therapies, were eligible for participation in this study. Patients were excluded if they had received prior bortezomib. Response was evaluated using the Bladé criteria. Treatment: Patients were randomized to either Arm A (bortezomib 1.3 mg/m2 on days 1, 4, 8, 11 every 21 days) or Arm B10 (bortezomib + mapatumumab 10 mg/kg on day 1 every 21 days) or Arm B20 (bortezomib + mapatumumab 20 mg/kg on day 1 every 21 days). Patients received a maximum of 17 cycles (1 year) and treatment was discontinued at any time for progressive disease or unacceptable toxicity. Subjects with complete response (CR) were treated for an additional 2 cycles after documentation of CR (not to exceed 17) and then followed to progression. This trial is registered with clinicaltrials.gov, number NCT00315757. Results. A total of 104 subjects were randomly assigned to the treatment arms. The median age of patients was 61.7 and mean prior therapies was 30.3% and 52.8% and the median duration of response was 8.5, 9.3

Table 1.
and 7.6 months, in arms A, B10 and B20, respectively (Table). Summary/Conclusions. Mapatumumab is a novel mAb that effectively engages TRAIL-R1. Encouraging preclinical observations led to a well designed randomized phase II clinical trial to determine safety and effectiveness of targeting TRAIL-R1 in MM. Our studies demonstrate that mapatumumab was well tolerated with no significant toxicity when added to bortezomib. However, in patients with rel/ref MM, mapatumumab adds cell-mediated cytotoxicity to bortezomib, further supporting the combination for the treatment of MM. TRAIL-R1 remains an important therapeutic target in cancer and mapatumumab is actively investigated in other malignant disorders, our data provide valuable insight into the tolerability and expected toxicity of two different doses of this novel drug. Detailed safety and efficacy analyses will be presented at the meeting.

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**ELOTUZUMAB IN COMBINATION WITH LENALIDOMIDE AND LOW-DOSE DEXAMETHASONE IN PATIENTS WITH RELAPSED MULTIPLE MYELOMA: A RANDOMIZED PHASE 2 STUDY**

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**Background.** Elotuzumab is a humanized monoclonal IgG1 antibody targeting human CS1, a cell surface glycoprotein. CS1 is highly and uniformly expressed on multiple myeloma (MM) cells, with limited expression on normal killer (NK) cells and CD8+ cells and little to no expression in most other normal tissues. Preclinical data indicate that the mechanism of action is primarily through NK-mediated antibody-dependent cellular cytotoxicity. In a MM xenograft mouse model, the anti-tumor activity of elotuzumab was enhanced by the addition of lenalidomide, significantly reducing tumor volume compared with either agent alone. A phase 1 study of elotuzumab plus lenalidomide and low-dose dexamethasone demonstrated an 82% response rate but did not identify a maximum tolerated dose in patients with relapsed/refractory MM. Results of proved durability, median time to progression not reached at a median follow-up of 12.7 months. Aims. To assess the efficacy and safety of elotuzumab in combination with lenalidomide and low-dose dexamethasone and determine the optimal dose of elotuzumab (10 mg/kg vs 20 mg/kg). Methods. Patients with relapsed/refractory MM who had received 1-3 prior therapies (excluding lenalidomide) were randomized to elotuzumab 10 mg/kg or 20 mg/kg IV (days 1, 8, 15, and 22 every 28 days in the first 2 cycles and days 1 and 15 of subsequent cycles), lenalidomide 25 mg PO (days 1-21) and dexamethasone 40 mg PO weekly. Prophylaxis for potential elotuzumab infusion-related AEs was methyprednisolone (50 mg IV), diphenhydramine (25-50 mg PO or IV) or equivalent, ranitidine (50 mg IV) or equivalent, and acetaminophen (650-1000 mg PO). Treatment continued until disease progression or unacceptable toxicity. All patients provided informed consent. Objective responses were assessed according to IWG criteria. Results. Among 63 enrolled patients (median age 63 years; range, 39-82), 57% had ≥2 prior therapies, 54% had prior bortezomib, 59% had prior thalidomide, and 52% had a β2 microglobulin level ≥5.5 mg/L. In total, 81% of patients had a partial response (PR) including 37% ≥ very good PR. Overall response rates (ORR) defined as ≥PR were 90% in the 10 mg/kg group (n=31) and 72% in the 20 mg/kg group (n=32). Results were similar irrespective of prior thalidomide, prior bortezomib, number of previous therapies, and β2 microglobulin. Median time to response was 30 days (range, 21-100). After a median follow-up of 4.9 months, median progression-free survival had not been reached; 9 (14%) patients have progressed. The most common grade 3/4 treatment-emergent AEs were neutropenia (14%), lymphopenia (14%), and thrombocytopenia (13%). The most common infusion-related AEs were grade 1/2 nausea (16%), dizziness (13%), headache (13%), and pyrexia (10%). One patient had a grade 3 infusion reaction AEs (rash). No patient withdrew due to an infusion reaction. Summary/Conclusions. Elotuzumab plus lenalidomide/dexamethasone resulted in rapid and high ORR in patients with previously-treated MM. The most common grade 3/4 treatment-emergent AEs were cytophenias. With premedication the incidence and severity of infusion reactions were low. Further clinical study of this combination using 10 mg/kg elotuzumab is warranted; a phase 3 trial is planned in 2011 (NCT01259797). Updated results will be presented at the meeting.

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**BORTEZOMIB(VELCADE®)+THALIDOMIDE-DEXAMETHASONE (VTD) IS SUPERIOR TO THALIDOMIDE-DEXAMETHASONE (TD) IN PATIENTS WITH MULTIPLE MYELOMA (MM) PROGRESSING OR RELAPSING AFTER AUTOLOGOUS TRANSPANTATION**

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In 2006, the EBMT and the IFM initiated a prospective, randomized, parallel-group, open-label phase III, multicenter study, comparing VTD (arm A) with TD (arm B) for MM patients in first progression/release after at least one autologous transplantation. TTP was the primary end point. Treatment was: bortezomib 1.3 mg/m2 as an i.v bolus on days 1, 4, 8 and 11, followed by a 10-day rest period (days 12 to 21), for 8 cycles (6 months) and then on days 1, 8, 15 and 22, followed by a 20-day rest period (days 23 to 42), for 4 cycles (6 months). In both arms, thalidomide was administered at 100 mg/day for 1 year and dexamethasone at 40 mg/day orally for 4 days every 5 weeks for 1 year. Response was assessed by EBMT criteria. Events were graded by the NCI-CTCAE, Version 3.0. Results. On 01/10/01, a first interim analysis based on 246 patients and 134 events was performed. The trial was then stopped because of superiority of VTD over TD. We report an updated analysis as of 02/12/10. 267 patients (135 in arm A, 132 in arm B) had been enrolled in the study and 157 events had been observed. The median age was 61 years (range 29-76). The stage according to the ISS was I in 56 %, II in 27 %, III in 17 %. The number of previous autologous transplants was one in 71 vs 69 patients and two or more in 64 vs 63 patients, in arms A and B respectively. The median follow-up was 27 months. The median TTP was 19.5 vs 13.8 months respectively in arms A and B, with a cumulative incidence of relapse/progression at 2 years of 56 vs 71 % (p=0.0011). The median PFS was 18.6 vs 12.7 months with a cumulative incidence at 2 years of 37% vs 23% (A vs B, p=0.0011). The OS in the first two years was 72 % vs 68% (p=0.18). The probability of achieving CR and CR+PR

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during the first year was 32% vs 12% and 90% vs 69% with VTD and TD (p=0.0001, and p=0.0001). In the VTD and TD arms, the mean number of treatment cycles for the first 8 cycles were 6.25 vs 6.88 and for the 12 cycles, 7.56 vs 9.93 respectively. Treatment was discontinued due to toxicity in 48 patients (VTD= 36, TD=12). 33 patients died during the treatment period (VTD= 14, TD= 19). The incidence of thromboembolic events >= grade 3 was similar in the two arms (6.6% vs 5.2%, p=ns, VTD vs TD) while >= grade 3 thrombocytopenia was higher with VTD (16% vs 7%, p= 0.025).

Conclusion. VTD resulted in significantly longer TTP and PFS in patients relapsing after ASCT with an acceptable toxicity. Protocol EU-DRACT number: 2005-001628-35.

LENALIDOMIDE AND DEXAMETHASONE (LEN + DEX) TREATMENT IN RELAPSED/REFRACTORY MULTIPLE MYELOMA (RRMM) PATIENTS DOES NOT INCREASE THE RISK OF SECOND PRIMARY MALIGNANCIES (SPM): ANALYSIS OF MM-009/010

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Background. Multiple myeloma (MM) is an incurable malignancy characterized by multiple relapses, and eventually, refractory disease and death. In a pooled update of the phase 3 MM-009/010 trial comparing Len + Dex vs placebo plus dexamethasone (PBO + Dex), Dimopoulos et al. (2009) reported that overall survival (OS) was significantly longer in patients treated with Len + Dex vs PBO + Dex (median of 38.0 vs 31.6 months, respectively; P = 0.045). However, with longer survival a greater number of SPMs may be observed. Cancer registry data (2003-2007) indicate that the incidence of all invasive cancers increases from 0.8 per 100 person-years in persons aged 55-59 to 2.2 per 100 person-years among persons 85+, respectively (Surveillance, Epidemiology, and End Results [SEER] 2010; myelodysplastic syndromes [MDS] not included). Moreover, this incidence may be higher in patients with MM as result of immune deficiency and underlying genetic predisposition.

Aim. This post hoc analysis of pooled MM-009/010 data was conducted to compare incidence rates (IRs) of SPMs between treatment arms and to compare these IRs with IRs of invasive cancers among similarly aged persons in the general population.

Methods. Potential SPMs were identified by review of medDRA terms under the “Neoplasm” System Organ Class. SPM incidence rates per 100 person-years were evaluated during active, double-blind treatment.

Results. The overall incidence of SPMs was low. There were 2 cases of MDS in the Len + Dex arm, and no cases of acute myeloid leukemia or B-cell malignancies in either arm. Non-melanoma skin cancers were noted in 14 Len + Dex patients and 2 PBO + Dex patients. Seven cases of solid tumors (5 with Len + Dex and 2 with PBO + Dex) occurred during double-blind treatment. IRs were similar between treatment arms. With an additional 1.5 average person-years during the extended follow-up phase, only one new SPM was identified in the Len + Dex arm, likely reflecting limitations in SPM ascertainment as only survival information was collected during this study phase.

Conclusions. With a median follow-up of 48 months, a significant OS benefit for Len + Dex treatment in RRMM patients was observed. The number of SPMs was low and IRs during active treatment (when medical surveillance could be expected to be most equivalent) did not differ between treatment arms. Importantly, IRs in both arms were comparable to the expected background incidence of invasive cancer among older individuals. The low number and type of SPMs seen did not change the benefit-risk profile for lenalidomide in RRMM patients, especially given the survival advantage seen with this therapy. The role of lenalidomide in the RRMM setting is confirmed as an effective treatment option.
Chronic myeloid leukemia - Clinical 2

1010
A NEW PROGNOSTIC SCORE (EUTOS SCORE) PREDICTING CYTOTOGENETIC RESPONSE AND PROGRESSION-FREE SURVIVAL IN 2060 PATIENTS WITH CHRONIC MYELOID LEUKEMIA ON IMATINIB TREATMENT

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Background. The outcome of chronic myeloid leukemia (CML) has been profoundly changed by tyrosine kinase inhibitors (TKI), but the prognosis of CML is still based on prognostic scores developed in the chemotherapy and interferon era. Aims. We analyzed for prognosis a multicentric multinational series of 2060 Ph+ BCR-ABL + CML patients who were treated front line with Imatinib or an Imatinib-based regime. Methods. First, we examined the relationships between the time of achieving a complete cytogenetic response (CCgR) during the first 18 months of therapy, the probability of achieving a CCgR later on, and the risk of progression to accelerated phase (AP) or blast phase (BP) after 18 months. Results. It was found that the most powerful predictor was the Cq status at 18 months, since only 31% of the pts who were not yet in CCgR at 18 mo, achieved a CCgR later on, and 23% of them progressed after 18 months. The whole series was then divided in a learning sample and a validation sample. The analysis of the learning sample, by logistic regression and chi-squared tests, identified spleen size (assessed by manual palpation and measured in cm below costal margin) and blood basophils percentage as the most significant prognostic variables. Using spleen size and basophils (7 x basophils + 4 x spleen size), a risk score could be assigned to all patients, and by the minimal p-value approach a "high risk" and a "low risk" group were identified (score more than 87 vs equal / less than 87). The score was validated in the validation sample. The positive predictive value of the new score was 34%, the sensitivity was 21%, and the specificity was 92%. Progression-free survival at 5 years was 90% (95% CI 88-92%) for high risk patients and 82% (95% CI 73-89%) for low risk patients. Conclusions. The power and the efficacy of TKIs are such that it is increasingly difficult to elaborate a prognostic system, but the new EUTOS score marks a significant improvement over prior, Sokal and Euro scores, and can be easily applied in clinical practice, until new biologic and molecular factors will be identified and shown to predict better the outcome and to select the treatment.

1011
EFFICACY AND SAFETY OF DASATINIB COMPARED WITH IMATINIB IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE (CML-CP): MINIMUM 24-MONTH FOLLOW-UP FROM THE DASISION TRIAL

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Background. In the phase 3 DASISION trial, first-line dasatinib showed higher and faster rates of complete cytogenetic response (CCyR) and major molecular response (MMR) compared with imatinib in patients with newly diagnosed CML-CP (Shah, Blood 2010; 116: abs 2009. Aims. Assess 2-year efficacy and safety in the DASISION trial. Methods. After informed consent, patients were randomized 1:1 to dasatinib 100 mg once daily (QD) (n=259) or imatinib 400 mg QD (n=260). Primary endpoint was confirmed CCyR (CCyR) rate (CCyR on two consecutive assessments) by 12 months. Results. Minimum 24-month follow-up will be presented. After 19.7 months, median follow-up (range 0.1-31.4), 81% and 80% of dasatinib-treated and imatinib-treated patients remained on therapy. 18-month cumulative response rates for dasatinib vs imatinib were: cCCyR 76% vs 70% (P=0.0566); CCyR 84% vs 78% (P=0.0932); and MMR 56% vs 37% (P=0.0001). MMR was more frequent with dasatinib in all Euro risk groups. BCR-ABL transcript levels (best levels ≤0.003% International Scale) occurred in 13% with dasatinib and 7% with imatinib (P=0.0119). Median time to CCyR or MMR calculated using competing risk analysis was shorter with dasatinib vs imatinib (CCyR: 3.2 vs 6.0 months; MMR: 15 months vs not yet reached). Transformation to accelerated/blast phase (AP/BP) occurred in six (2.5%) dasatinib-treated vs nine (3.5%) imatinib-treated patients; one additional patient transformed 183 days after discontinuing dasatinib; no patient achieving MMR transformed. Protocol-defined progression (death from any cause, transformation to AP/BP, increasing WBCs, loss of complete hematologic response or major cytogenetic response) occurred in 15 patients (5.8%) in each arm. For dasatinib vs imatinib, 18-month overall survival rates were 96% vs 93%, progression-free survival rates (no transformation to AP/BP or loss of response) were 95% vs 94%, failure-free survival rates (ELN 2006 criteria) were 93% vs 90%, and maximum clinical benefit rates (no progression, failure, or intolerance) were 87% vs 86%. Longer follow-up is needed and 5-year follow-up is planned. Protocol-defined progression (death from any cause, transformation to AP/BP, increasing WBCs, loss of complete hematologic response or major cytogenetic response) occurred in 15 patients (5.8%) in each arm. For dasatinib vs imatinib, 18-month overall survival rates were 96% vs 93%, progression-free survival rates (no transformation to AP/BP or loss of response) were 95% vs 94%, failure-free survival rates (ELN 2006 criteria) were 93% vs 90%, and maximum clinical benefit rates (no progression, failure, or intolerance) were 87% vs 86%. Longer follow-up is needed and 5-year follow-up is planned. Drug-related nonhematologic adverse events (AEs; any grade) in ≥10% of patients (dasatinib vs imatinib) were fluid retention (23% vs 43%); including superficial edema: 10% vs 36%, pleural effusion: 12% vs 0%, diarrhea (18% vs 19%), nausea (9% vs 21%), vomiting (5% vs 10%), myalgia (32% vs 2%), fatigue (8% vs 11%), headache (12% vs 10%), and rash (11% vs 17%); grade 3/4 rates for these AEs were ≤1%. Only dasatinib-treated patients experienced pleural effusion (2% grade 1, 9% grade 2, ≤1% grade 3), which did not seem to impact efficacy. Grade 3/4 cytopenias with dasatinib vs imatinib were: anemia, 11% vs 7%; neutropenia, 22% vs 20%; and thrombocytopenia, 19% vs 10%. Most cytopenias (75%) occurred within 4 months of treatment. Grade 3/4 lab abnormality rates were ≥3% in both arms, except hypophosphatemia (dasatinib 5%, imatinib 24%). For dasatinib vs imatinib, 56% vs 59% had dose interruption, 25% vs 14% had dose reduction, and 6% vs 4% discontinued due to AEs. Conclusions. After 18 months, dasatinib continues to show significantly higher CCyR and MMR rates over imatinib and remains generally well tolerated, supporting first-line dasatinib use in newly diagnosed CML-CP.

1012
TREATMENT OF CHRONIC PHASE (CP) CHRONIC MYELOID LEUKEMIA (CML) PATIENTS WHO HARBOR THE BCR-ABL T315I MUTATION WITH SUBCUTANEOUS OMACETAXINE RESULTS IN IMPROVED SURVIVAL COMPARED TO HISTORICAL DATA

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Background. Point mutations in the ABL kinase domain are emerging as the most frequent cause of drug resistance in CML with the specific T315I point mutation conferring the highest level of resistance to all approved TKIs. The T315I mutation is associated with disease progression and a poor prognosis. In the largest retrospective study of the natural history of T315I+ CML patients in CP, the median survival was only 22.4 months with a survival rate of 71% at one year from time of mutation detection (Nicolini et al., 2009). New therapies, particularly those that are independent of Bcr-Abl inhibition, are needed to provide a solution for this “Achilles heel” in the TKI armamentarium and prolong survival in T315I patients. Aims. To evaluate the safety and efficacy of subcutaneously (SC) administered omacetaxine in imatinib-resistant T315I+ CML-CP patients. Methods. 62 adult CML-CP patients, who signed an informed consent, harboured the T315I mutation as confirmed by direct sequencing or DHEIC in a central laboratory, and demonstrated hematologic or cytogenetic resistance to imatinib therapy were enrolled in this study making it the largest prospective study in this patient population worldwide. Induction treatment con-
sisted of 1.25 mg/m² SC omacetaxine bid for 14-days every 28 days until hematologic improvement, followed by maintenance schedule of 1.25 mg/m² SC omacetaxine bid for 7-days every 28 days and adjusted for tolerance. The primary efficacy endpoints were the achievement of complete hematologic response (CHR) and major cytogenetic response (MCyR) and a key secondary endpoint was median overall survival. Results. For the 62 CML-CP Patients enrolled the median age at entry was 59 yrs (26-83) with 69% male and a median disease duration of 51 months (13-254). All patients failed prior imatinib therapy, and 75% failed two or more prior TKIs (24% failed 3 or more TKIs). The median follow-up time was 19.1 months (1-48). CHR was achieved in 47 (76%) patients with a median duration of 8.9 months (1.5-43.6+) and MCyR was achieved in 24% patients (11 complete and 4 partial) with a median duration of 6.5 months (2.1-29+). At the latest follow-up the median overall survival time was not reached and the survival rate at 24 months was 65.2% (Figure 1). Grade 3/4 related events occurred in 52/62 (84%) of patients. The most commonly reported events were thrombocytopenia (72%), anemia (44%) and neutropenia (56%). Non-hematologic toxicities were generally grade 1/2 with the most frequently reported; diarrhea (56%), pyrexia (27%), fatigue (25%), asthenia (25%) and nausea (24%). Treatment delays occurred in approximately 75% of the patients with median duration of 11 days (3-81). The primary causes of delay were; thrombocytopenia, neutropenia, pancytopenia and patient availability. Nine deaths occurred during the study. Three of these were considered to have a possible relationship to omacetaxine: sepsis, pancytopenia, and sudden death with unknown cause. Summary. Omacetaxine administration produces durable hematologic and cytogenetic responses with a safety profile primarily comprising of hematologic toxicities. The overall survival of T315I+ CML-CP patients treated with omacetaxine exceeds the survival reported in the literature.

**1013**

**KIR2DS1 GENOTYPE IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO CML AND POOR RESPONSE TO IMATINIB**

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**Background.** Killer immunoglobulin-like receptors (KIR), expressed on the surface of natural killer (NK) cells, play an important role in determining NK function and cytotoxicity. NK cells are important in tumour-cell killing and the inherited KIR-repertoire has been shown to influence tumour susceptibility. Aims. As NK cells can kill chronic myeloid leukaemia (CML) cells including the leukaemia-initiating-cell (LIC), we investigated if KIR-genotype influenced susceptibility to CML development and response of CML patients to treatment with imatinib. Methods and Results. Comparison of 190 CML patients in 1st chronic-phase (CP) to 161 healthy-controls revealed over-representation of KIR2DS1 in patients, 59% (121/201) vs 47.5% (69/161); OR 1.607 (P=0.05). We then investigated if KIR2DS1 genotype in CML patients predicts for response to imatinib treatment. NK cells are expanded in CML patients on tyrosine kinase inhibitors (TKI). We evaluated the impact of KIR genotype on the outcome of 166 patients with CML-CP receiving first-line imatinib treatment. KIR2DS1 positive patients had significantly lower 2-year probabilities of CCyR (82.3% and 65.6%, p=0.05), PFS (98.4% vs 91.0%, p=0.01 respectively) and OS (100% vs 92.6% p=0.03 respectively) than KIR2DS1 negative patients. Multivariate analysis revealed KIR2DS1 genotype (RR=0.66, p=0.03) and Sokal-risk-score (low RR=1, intermediate RR=0.65, p=0.04 and high RR=0.59, p=0.04) to be the only independent predictors for CCyR. Furthermore, KIR2DS1 was the only independent predictor for both PFS (P=0.02) and OS (P=0.03). The association between KIR2DS1 and outcome was validated in 174 CML-CP patients treated in the multi-center SPIRIT-I trial. KIR2DS1+ patients (n=66) had a lower probability of achieving CCyR and lower PFS and OS than KIR2DS1- patients (n=100) (76.9% vs 87.9%, p=0.004, 85.3% vs 98.1% p=0.01 and 94.4% vs 100%, p=0.02 respectively). Again on multivariate analysis KIR2DS1 remained the only independent predictor for all three outcomes. Because KIR2DS1 interaction with group 2 HLA-C molecules on target cells could theoretically inhibit an activating signal mediated by KIR2DS1, we hypothesized that any effect of KIR2DS1 would be greatest among individuals who are missing group 2 HLA-C ligand for KIR2DL1. We determined the various combinatorial frequencies of KIR2DS1 with group 2 HLA-C alleles in the discovery plus validation patients (n = 340). The impact of KIR2DS1 on CCyR was more significant when the ligand for the corresponding inhibitory receptor, KIR2DL1 was absent; the 2-yr CCyR rate for KIR2DS1-/KIR2DL1+/C2+, KIR2DS1+/KIR2DL1+/C2+ and KIR2DS1+/KIR2DL1+/C2- patients were 98.6%, 94.7%, and 64.1% respectively (P=0.00006). The mechanism by which KIR2DS1 increases susceptibility and affects outcome in patients with CML-CP on imatinib is unclear. KIR2DS1+ NK-cells secrete TGF-beta following ligand interaction, which can inhibits Akt signalling, a suppressor of FOXO3a, in CML-LIC. This may represent an important mechanism for imatinib resistance. Alternatively, KIR2DS1 may be simply a surrogate marker for genes directly involved in CML pathogenesis. Conclusion. In conclusion, our data demonstrate that KIR2DS1 is over-represented in patients with CML and may predict response to imatinib and identify those patients at greater risk of treatment failure.
Acute myeloid leukemia - Clinical 2

1015
RISK OF HEMATOLOGICAL MALIGNANCIES AMONG FIRST-DEGREE RELATIVES OF PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND MYELODYSPLASTIC SYNDROMES (MDS) - A POPULATION-BASED STUDY IN SWEDEN

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Background. Apart from rare pedigrees with multiple cases of AML there is limited data on the extent of familial aggregation in AML and MDS in the population. In a comprehensive population-based study, we estimated risk of AML, MDS, other hematological malignancies and solid tumors combined among first-degree relatives of AML and MDS patients compared to first-degree relatives of matched controls. Design and Methods. Swedish population-based registry data were used to evaluate outcomes in 20,579 first-degree relatives of 6,962 AML patients (diagnosed 1958-2004; median age 64 years) and 3,994 first-degree relatives of 1,388 MDS patients (diagnosed 1993-2004; median age 76 years) compared with 90,406 first-degree relatives of 37,384 and 15,818 first-degree relatives of AML and MDS patients, respectively. Using a marginal survival model, we calculated relative risks (RR) and 95% confidence intervals (CI) as measures of familial aggregation. Results. AML and/or MDS did not aggregate significantly in relatives of patients of AML, MDS or in the combined group (Table). The risk of polycythemia vera (PV) was significantly higher in relatives of AML patients compared to controls but the increased risk of myeloproliferative neoplasms as a group did not reach statistical significance. Lymphoid malignancies showed some increased risk in relatives of AML patients (with a borderline significant increased risk of chronic lymphocytic leukaemia; CLL) but not in relatives of MDS patients. The global risks for any hematopoietic or solid tumor were significantly but modestly increased among relatives of AML patients. When analysing risks in relatives of younger patients (≤ 20 years at diagnosis) with AML a significantly increased risk (RR 7.53; CI 1.25-45.13) of AML/MDS and a 3.01-fold RR (CI 1.09-8.31) for all myeloid malignancies combined was ob-

Table 1.
served. Among lymphoid malignancies, there was a significantly increased risk of multiple myeloma (RR 5.02; CI 1.25-20.11) but the numbers are small. All hematopoietic cancers combined were significantly increased (RR 1.85; CI 1.07-3.18) as well as all solid tumors combined (RR 1.27; CI 1.05-1.52). In general, despite the small sample size of younger patients, the relative risks were higher in this group than among all AML patients. Conclusions. The lack of familial aggregation of AML or MDS is striking and in sharp contrast to findings in patients with other myeloid and lymphoproliferative disorders. However, relatives of young patients do seem to be at increased risk of AML/MDS and other hematopoietic malignancies suggesting that they share a genetic susceptibility. Interestingly there is an increased risk of PV and small but significantly increased risks of any hematopoietic or solid cancer among relatives to AML patients. The results are important since many patients worry about a potentially increased risk of their family members to develop AML or MDS and many clinicians are of the opinion that there is a small but significant familial pathogenetic component. The increased risk of PV, any hematopoietic, any solid tumor and CLL among relatives of AML patients may point to the existence of broadly shared germ line susceptibility genes and/or environmental factors.

1016 INTEGRATIVE PROGNOSTIC RISK SCORE IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NORMAL KARYOTYPE AGED 18-60 YEARS

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Background. Prognostic risk factors have been described in AML including cytogenetics, WBC count, and response to induction chemotherapy and have been used extensively to stratify treatment. Patients with AML and normal karyotype (CN-AML) have usually been classified as intermediate risk. Many efforts have been made to identify genetic mutations (e.g. FLT3, NPM1, CEBPA, MLL, NRAS, IDH1/2, and WT1) that allow further sub-classification of CN-AML patients and possibly risk-directed therapeutic intervention. In addition to mutations, modulated expression of genes important for proliferation, and differentiation have also been shown to be predictive for CN-AML patient outcome (e.g. MN1, BAALC, ERG, ID1, WT1). This molecular heterogeneity of CN-AML is not fully reflected in current classification systems. Aims. To integrate available clinical and molecular information for CN-AML patients into one risk score.

Patients and Methods. 275 CN-AML patients from multicenter treatment protocols of the AML Study Group Germany (AMLSG) were included. The AMLSG protocol 0198, a CN-AML protocol, included these patients. Diagnostic samples from 799 patients who were intensively treated on AMLSG trials [AML HD98A (n=729), APL HD95 (n=70)] were analyzed for the presence of NPM1, FLT3 (ITD, TKD), CEBPA, IDH1/2, RUNX1 and TET2 mutations by standard PCR-based methods. Results. ASXL1 mutations were detected in 54 (6.8%) of 799 pts. All mutations were heterozygous. The most frequent mutation (38/54; 70%) was an insertion of guanine at codon

1017 ASXL1 MUTATIONS IN ACUTE MYELOID LEUKEMIA: RESULTS ON 799 PATIENTS TREATED WITHIN THE AML STUDY GROUP (AMLSG)

P Paschka,2 R Schlenk,3 T Aultzik,2 V Gaidzik,1 M Habdkan,1 A Cobacioglu,1 L Bullinger,1 D Spåth,1 CH Kühne,1 A Kündgen,1 M von Lilienfeld-Toal,1 G Held,2 HA Horst,1 M Rummel,1 S Wilhelm,1 H Döhner,1 K Döhner3
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Background. The ASXL1 (Additional Sex Comb-Like 1) gene on chromosome 20q11.1 encodes a protein believed to be involved in chromatin modification and to act as a co-activator for the retinoic acid receptor. ASXL1 mutations occur with high incidence in CMMLL (40%) and less frequently in AML, CML, MDS and myeloproliferative neoplasia. They can be classified into 12 and are mainly frameshift mutations creating a premature stop codon. A recent AML study reported on unfavorable impact of ASXL1 mutations on induction success and overall survival (OS) (Chou et al., Blood 2010;116:4086-94). Aims. To analyze the incidence, clinical, cytogenetic and molecular features of ASXL1 mutations in younger (18-60 years) AML patients and to assess their prognostic relevance. Methods. Diagnostic samples from 799 patients who were intensively treated on AMLSG trials [AML HD98A (n=729), APL HD95 (n=70)] were analyzed for the presence of ASXL1 mutations. Hot spots of ASXL1 exon 12 were screened for mutations using GeneScan fragment analysis of PCR products followed by direct sequencing. Patients were also assessed for the presence of NPM1, FLT3 (ITD, TKD), CEBPA, IDH1/2, RUNX1 and TET2 mutations by standard PCR-based methods. Results. ASXL1 mutations were detected in 54 (6.8%) of 799 pts. All mutations were heterozygous. The most frequent mutation (38/54; 70%) was an insertion of guanine at codon
Conclusions. A survival advantage was observed for patients with a decreased probability of cure (+1). These preliminary data suggest that quizartinib achieves meaningful reductions in marrow blasts in a substantial proportion of pts with both refractory and relapsed FLT3-ITD+ AML, and many of these pts were successfully bridged to HSCT. These encouraging efficacy results and an acceptable safety profile in this high risk population support continued clinical evaluation.
Myeloproliferative disorders - Clinical

1020
A RANDOMIZED STUDY OF JAK INHIBITOR RUXOLITINIB (INC424) VS BEST AVAILABLE THERAPY IN PRIMARY MYELOFIBROSIS (MF), POST-POLYCYTHEMIA VERA-MF OR POST-ESSENTIAL THROMBOCYTHEMIA MF

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Background. Dysregulated JAK-STAT signaling is a key feature of myelofibrosis (MF), a myeloproliferative neoplasm associated with splenomegaly, constitutional symptoms and shortened survival. Overall median survival is 2.25-4 years for high- and intermediate risk-2 patients, respectively as defined by IWG-MRT criteria (Cervantes et al., Blood 2009). Approximately half of MF patients carry a gain-of-function mutation in the Janus kinase (JAK) 2 gene (JAK2V617F); dysregulation of the JAK pathway occurs regardless of JAK2V617F status and contributes to disease pathophysiology. There are currently no effective drug therapies for MF. Ruxolitinib is a selective JAK1 and JAK2 inhibitor with clinical activity in MF. Aims. COMFORT-II, a randomized (2:1), open-label, phase 3 study measured the efficacy, safety, and tolerability of ruxolitinib given twice daily compared to best available therapy (BAT), which could include no therapy or other agents, in adult patients with high- or intermediate risk-2 primary MF, post polycythemia vera MF or post essential thrombocytemia MF with palpable splenomegaly. The primary efficacy endpoint is the proportion of subjects achieving ≥35% reduction in spleen volume from baseline to week 48 as determined by magnetic resonance imaging (MRI) or computed tomography (CT) and analyzed using the Cochrane-Mantel-Haenszel test stratified by baseline risk category. For the 219 subjects enrolled, the power of two-sided CMH test with alpha level of 0.05 would be 93.7% assuming that the ratio of subjects with baseline prognostic category of intermediate- vs. high-risk was 1:1, response rate for intermediate-risk for active and control treatment groups was 40% and 15%, respectively, and for high-risk 30% and 5%, respectively. The key secondary endpoint is the proportion of subjects achieving ≥35% reduction in spleen volume (by MRI or CT) from baseline to week 24, while other secondary endpoints include duration of maintenance of reduction in spleen volume, time to achieve a first ≥35% reduction, progression-free survival, leukemia-free survival, overall survival and change in bone marrow histomorphometry. Patients were enrolled at 56 sites in Europe and the UK from July 2009 until January 2010. Primary analysis will occur in March 2011. Results. A total of 219 patients were randomized; 146 to ruxolitinib and 73 to BAT. Results for the primary and secondary endpoints will be reported. A summary of the most common adverse events in the ruxolitinib vs BAT arms will be reported as was the rate of treatment discontinuation. Summary/Conclusions. The results of COMFORT-II will provide important information that may result in a new standard of care for a large number of patients with myelofibrosis. A companion study, COMFORT-I, has met its primary and symptom assessment secondary endpoints. COMFORT-I enrolled 309 patients with similar eligibility criteria from the US, Canada, and Australia, and compared ruxolitinib to placebo with a primary endpoint of response defined as the percentage of patients achieving 35% or greater reduction in spleen volume at 24 weeks. It will be important to compare the results of these 2 studies that differ in their comparator arm and the timing of response assessment.
Background. The JAK2V617F mutant enzyme has been linked to the pathogenesis of myeloproliferative neoplasms. SB1518 is a potent inhibitor of JAK2 and its JAK2V617F mutant. In previously reported Phase 1 studies, patients with myelofibrosis (MF) who received SB1518 achieved steady-state plasma levels above the IC50 for JAK2 at all doses (100-600 mg/d). Target inhibition, measured by phosphorylation of the STAT3, STAT5, and JAK proteins in PBMCs and whole blood, was also shown at all doses. 400 mg/d was chosen as the recommended dose (RD) for Phase 2 study. Aim. To assess the spleen response rate, defined as a ≥5% reduction in MRI-measured spleen volume between baseline and Week 24. Methods. Qualifying patients had primary, post-ET, or post-PV MF, palpable splenomegaly ≥5 cm below the left costal margin, and were not suitable for standard therapy. Based on minimal myelosuppression in the Phase 1 trial, no minimal hematology values were required for study inclusion. Each patient received daily oral SB1518 in ongoing 28 day cycles. Disease symptoms were evaluated using the MF-SAF. Results. Patients: In this ongoing trial, thirty-four MF patients (median age 58.5; range, 44-84 years) were consented and enrolled, of which 25 (74%) were men. Median age at inclusion was 64 years (29-82) (PV=65 (29-82), ET = 62 (50-77)). Period between diagnosis and inclusion was 2.8 years (0.27,4) (PV= 3.1 (0.27,4), ET=2.2 (0.01-22.6). Treatment prior to vorinostat included hydroxyurea (53%) (PV=43%, ET=75%), interferon -alpha (8%) (PV=11%, ET=0%), anagrelide (12%) (PV=6%, ET=27%) and busulfan (4%) (PV=3%, ET=8%). Of the 14 patients (6 PV, 6 ET) evaluable for clinical responses are achieved in a high proportion of patients adhering to treatment. After months of treatment vorinostat has generally well-tolerated with minimal AE's and hematologic toxicity. An early drop-out was observed for MF associated symptoms, including abdominal pain, cough, and night sweats. Conclusions. SB1518 shows promising efficacy in alleviating MF-associated splenomegaly and constitutional symptoms at a dose that induces minimal myelosuppression. Once-daily dosing is well tolerated, with manageable GI toxicity as the main side effect. Given the lack of myelosuppression with SB1518, this JAK2 inhibitor is of particular importance for MF patients with impaired hematopoiesis.

Background. Conventional agents used in the management of polycythemia vera (PV) and essential thrombocythemia (ET) include hydroxyurea and in younger patients alpha-interferon or anagrelide as useful alternatives. Since all of these agents have side effects besides potential leukemogenic potential for hydroxyurea several clinical trials have been conducted to find alternative and better drug formulations. Histone deacetylase inhibition (HDACi) has been shown to impair the autonomous proliferation of haematopoietic cells of PV and ET patients carrying the JAK2 V617F mutation. Aims. The present study evaluates the efficacy and safety of vorinostat in the treatment of PV and ET in a non-randomized, open-label ongoing phase II study. Methods. Fifty-nine pts. (17 ET, 39 PV) from whom informed consent was obtained were included and given 400 mg of vorinostat daily for 6 months. Results. We report preliminary data for 59 pts, 70 % PV (m:f=53%/47%), 30 % ET (m:f=58%/62%) with a median follow-up from start of vorinostat of 8 weeks (range=0-36, IQR=4,28). Median age at inclusion was 64 years (29-82) (PV=65 (29-82), ET = 62 (50-77)). Period between diagnosis and inclusion was 2.8 years (0.27,4) (PV= 3.1 (0.27,4), ET=2.2 (0.01-22.6). Treatment prior to vorinostat included hydroxyurea (53%) (PV=43%, ET=75%), interferon -alpha (8%) (PV=11%, ET=0%), anagrelide (12%) (PV=6%, ET=27%) and busulfan (4%) (PV=3%, ET=8%). Of the 14 patients (6 PV, 6 ET) evaluable for clinical haematological response after visit 11 (completion of protocol), 4 achieved complete response (CR), 6 achieved partial response (PR) and 4 achieved no response (NR) identifying a response-rate of over 70%. Adverse effects (AE's) reported at visit 11 were all grade 1 and included constipation (1 pt), weight loss (1 pt), fatigue (1 pt), hyperglycemia (1 pt) and mucositis (1 pt). The most common AE's during the treatment period were gastrointestinal (anorexia, nausea, vomiting, diarrhea) typically grade 1/2, manageable and improving within months. Seventy-eight percent experienced hair loss, 1 pt grade 3. Fourteen percent experienced renal toxicity (grade 1/2) and 7% liver toxicity (unknown grade). Other grade 3/4 non-hematologic toxicities were anorexia (14%), nausea (7%), diarrhea (7%), fatigue (7%), dry mouth (7%). Fifty-seven percent required at least one dose reduction. An additional 11 pts. dropped out before visit 11 due to AE's (no: 4), serious AE's (no: 4) and unknown (no: 3). Serious AE's included 1 pt. with a deep vein thrombosis, 2 pts. with thrombocytopenia (1 grade 3, 1 unknown) and 1 pt. due to renal toxicity. Of the 11 pts. 2 achieved CR, 2 achieved PR, 6 achieved NR as best response before dropping out of protocol. Further up-dating will be at EHA. Conclusion and perspectives. Vorinostat is effective in PV and ET patients. Clinical responses are achieved in a high proportion of patients adhering to therapy. After months of treatment vorinostat is generally well-tolerated with minimal AE's and hematologic toxicity. An early drop-out rate of 44% draws attention to side effects which in future trials may be diminished by dose reduction or combination therapy (eg. hydroxyurea, interferon-alpha or JAK2-inhibitors).
ASXL1 MUTATIONS IN PATIENTS WITH MYELOFIBROSIS.

Background. Additional sex comb-like 1 (ASXL1) gene (chr 20q11.1) belongs to the Enhancer of Trithorax and Polycomb gene family. It functions as dual transcriptional activator/suppressor including repression of retinoic acid receptor-mediated transcription. Mutations in ASXL1 were recently demonstrated in a spectrum of chronic- and blast-phase myeloproliferative neoplasms (MPN). Aims. The current study seeks to determine ASXL1 mutational frequency and clinical correlates in a large series of patients with myelofibrosis (MF). Methods. We investigated ASXL1 mutational status of 290 patients with MF including 166 PMF, 36 PPV and 28 PET. Somatic mutations of ASXL1 were identified by sequencing exon 12 of whole-genome amplified DNA isolated from granulocytes; all mutations were validated by re-sequencing genomic DNA from the archival sample. Mutational status was correlated with clinical and biological features. Results. At the time of writing, results were fully available for 117 of 230 patients; details of the whole series will be presented at the meeting. A total of 21 different mutations were identified in 36 patients (31%), 24 out of 64 with PMF (38%) and 12 out of 53 (23%) with PPV/PET-MF. All mutations were heterozygous deletions, missense or nonsense mutations presumed to truncate the plant homeodomain finger domain. Sixty-four percent of ASXL1 mutated patients were JAK2V617F-positive and one patient was MPLW515L mutated. The frequency of ASXL1 mutations was similar in JAK2V617Fpos (30%) and JAK2V617Fneg (32%) cases. Median V617F allele burden was 48.6±26.7% in ASXL1/JAK2V617Fpos vs 57.9±24.4% in ASXL1wildtype/JAK2V617Fpos patients. Screening of EZH2 and IDH1/2 mutations in the same cohort showed that ASXL1, EZH2 and IDH1/2 are not mutually exclusive events. In fact, mutations of EZH2 and ASXL1 were simultaneously present in 6 cases. IDH1/2 mutations were found in 3 cases, two of whom showed concomitant ASXL1 mutation. ASXL1/IDH1-2 mutations were detected at chronic phase in both patients who later developed acute leukemia. Seven patients showed ASXL1 mutation as the sole molecular abnormality. There was no significant difference between mutated and unmutated patients as concerns age, sex distribution, clinical characteristics, leukemia transformation and overall survival. However, ASXL1 mutations were found to be preferentially associated with an abnormal karyotype; the latter occurred in 10 of 17 mutated patients (59%) compared to 7 of 29 unmutated patients (24%) (P=0.02). Conclusions. Preliminary data in 117 patients with MF analyzed for ASXL1 mutations discovered a high mutational rate in both primary and post-PV/post-ET MF. ASXL1 mutations did not associate with an unique phenotype nor deserved a detrimental effect on survival. However, the high frequency (52%) detected in JAK2V617F-negative subjects suggests that ASXL1 genotyping may be of help in the routine diagnostic path in MF patients. (A project of AGIMM supported by AIRC, Italy)
The clonal advantage of del(5q) MDS stem cells is mediated by increased adhesion to the microenvironment


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Background. The myelodysplastic syndromes (MDS) constitute a heterogeneous group of clonal disorders of hematopoietic stem cells (HSC) leading to ineffective hematopoiesis in one or more lineages in the bone marrow. The most frequent cytogenetic entity is the 5q-syndrome, characterized by loss of the 5q13-33 region. Lenalidomide has emerged as a very effective therapy for this subgroup of patients. Its mechanism of action, however, has hitherto remained elusive. Interestingly, the more primitive hematopoietic compartment seems to possess a clonal advantage where del(5q) HSC are able to outcompete normal HSC. We have previously demonstrated that lenalidomide is able to abrogate this clonal advantage and found that lenalidomide restored expression of the matricellular protein SPARC, a gene located within the commonly deleted region on chromosome 5q. We hypothesized that the decreased expression of SPARC in del(5q) HSC leads to increased adhesion of HSC to their respective niche cells, translating to increased survival, partly explaining the competitive advantage against non-del(5q) HSC. Aims. We conducted a study to analyze the effect of lenalidomide on the HSC/progenitor compartment in del(5q) MDS in order to test whether an hematopoietic stem cell (HSC)-intrinsic decrease of SPARC explains the why and how a clone of cells inherently defective at spawning functioning cellular descendants is not selected against, but rather exhibits a clonal advantage. Methods. We analyzed cell cycle distribution, frequency of apoptosis, and expression of adhesion markers on normal and del(5q) HSPC by multi-parameter flow cytometry. We analyzed the adhesion of normal and del(5q) HSPC to defined matrix components of the microenvironment - fibronectin and VCAM-1. We overexpressed SPARC by lentiviral transduction in HSPC and analyzed the effect on engraftment in NSG-mice. Results. Multiparameter flow cytometry revealed a slight increase in proliferation of del(5q) versus normal HSC. Patients treated with lenalidomide exhibited complex changes in their expression of adhesion markers. In functional adhesion studies we observed that HSPC from del(5q) patients exhibited stronger adhesion than normal bone marrow cells to fibronectin and VCAM-1. Recombinant SPARC protein abrogated adhesion to VCAM-1 specifically in a subset of patients, while having no significant effect on normal HSPC. Overexpression of SPARC led to severely reduced engraftment in NSG-mice. Summary/conclusion. These studies suggest that decreased expression of SPARC leads to increased adhesion of del(5q) HSC/progenitor cells to defined components of the microenvironment and may explain why del(5q) HSC are able to outcompete the remaining healthy HSC. Our studies implicate that lenalidomide is able to abrogate this clonal advantage partly via its increase in SPARC expression with a consequent decrease in adhesion.

Gene mutations of the telomerase complex in patients presenting with refractory cytopenia of childhood (RCC) - do we need to know?

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Refractory Cytopenia of Childhood (RCC), the most common form of myelodysplastic syndrome (MDS) in childhood, frequently presents with a hypocellular bone marrow. Thus, inherited bone marrow failure (BMF) disorders are an important differential diagnosis. In particular, dyskeratosis congenita (DC), one of the most common types of IBMF, has to be taken into account. Because RCC and DC cannot be distinguished by hematopoietic potential, accurate clinical examination is mandatory. However, subtle clinical characteristics associated with DC as well as the overgrowth of abnormal megakaryocytes make the differential diagnosis difficult. Our group reported on two patients with mutations in the gene of the human telomerase RNA component (TERC) who had been diagnosed with RCC in the absence of obvious clinical signs of DC. We hypothesized that a number of children presenting with hypocellular RCC may in fact suffer from DC identifiable by mutational analysis of the genes of the telomerase complex - namely, DKC1, TINF2, NOLA2, ALAS2, TERC and TERT. We therefore performed mutational screening for these 6 genes in 100 consecutive German patients enrolled in the prospective study EWOG-MDS-98 with primary hypocellular RCC without constitutional aberrations or myelofibrosis. The two patients with known TERC mutations were included in this series. In both patients with TERC mutation previously published, we uncovered six patients with mutations in the 6 genes studied. One boy carried a mutation in DKC1, two patients had a TINF2 mutation and another patient harboured a mutation in TERT. No further TERC mutations were identified. All mutations detected were heterozygous, and, with the exception of one child with a TINF2 mutation, all aberrations proved to be of germline origin. Five of the six patients carried known mutations, while the patient with TERT mutation exhibited a novel alteration. None of these patients had clinical signs of DC at time of diagnosis of RCC. The boy with DKC1 mutation had stable low blood counts over several years and died of pulmonary fibrosis. Both children with TINF2 mutation underwent HSCT; the child with the somatic mutation in hematopoietic cells remains in stable condition, while the other patient died from severe skin and pulmonary GVHD. One of the two children with TERC mutation died of CMV reactivation after HSCT, while the other child with TERC mutation and the patient with TERT alteration showed decreasing blood counts in the absence of HSCT. In summary, 6% of phenotypically normal children with RCC were shown to carry germline or somatic mutations in one of 6 genes of the telomerase complex. At least one of the identified individuals had an unusually complicated course after HSCT. In view of the excellent outcome of RCC in children with RCC, we suggest that it is important to identify patients with telomerase complex mutations. We currently perform telomere length analyses on granulocytes from the 100 patients to determine whether there is a significant difference in telomere length between RCC cases with or without telomerase complex mutations. Telomere length may provide a diagnostic tool for rapidly identifying RCC patients with mutations in one of these genes.
ABC7 expression plays a key role in mediating the erythroid failure, aberrant ALAS2 and FTMT expression and mitochondrial iron accumulation in acquired RARS. Moreover, up-regulation of ABCB7 restores RARS erythropoiesis and reverts ALAS2 and FTMT expression towards the normal range. In the absence of ABCB7 mutations or hypermethylation, upstream events are currently being investigated.

EXOME SEQUENCING IDENTIFIES THE MPL GENE AS A CAUSE OF FAMILIAL APLASTIC ANAEMIA

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Background. The primary cause of many paediatric diseases remains elusive; however in some cases they may represent recessive single gene disorders. The problem is therefore how to identify a gene defect in a particular individual. To this end, exome sequencing has become the method of choice. One problem with this methodology is that the discriminatory power often lies in comparing unrelated individuals with the same disease to identify a common causative variant. This depends on the disease being genetically homogenous, but this will not always be the case. An example is aplastic anaemia (AA) which can be "labelled" as idiopathic, acquired or constitutional. Although mutations have been described in some genes, these only account for a small proportion of cases of AA. Aims. From our collection of uncharacterised AA patients we selected a consanguineous family with severe AA in two siblings. In order to characterize this family one of the affected sibs had the entire exome sequenced. The disease causing gene identified in this family would then be screened in other uncharacterized AA patients with severe AA in two siblings. In order to characterize this family one of the affected sibs had the entire exome sequenced. The disease causing gene identified in this family would then be screened in other uncharacterized AA patients.

Methods. Genomic DNA from an affected individual was hybridised to a Nimblegen exome library before being sequenced on a GAIIx genome analyzer. Sequencing data were processed and by comparing to the reference human genome sequence and the 1000 genomes project, unique homozygous changes were identified. Biologically relevant changes were confirmed by Sanger sequencing. After confirming segregation in the rest of the family, the MPL gene was screened for mutations in 33 index cases with AA (<13 years) using denaturing HPLC. Any abnormal traces were confirmed by direct sequencing. Results. A homozygous mutation c.1248 G>A, p.Trp416Stop in the thrombopoietin receptor gene, MPL, was identified by exome sequencing and was shown to segregate with the disease (patient 1). This is a novel mutation occurring in a gene that has been previously associated with congenital amegakaryocytic thrombocytopenia (CAMT) - a rare autosomal recessive bone marrow failure syndrome characterised by early onset of isolated hypomegakaryocytic thrombocytopenia that often evolves to affect all three marrow lineages. We then identified two further novel mutations in two different patients from our screen of 33 index cases with AA (patients 2 and 3, Table 1). Interestingly, in patient 3 with the Pro394Ser mutation, the mutant T allele is present at a reduced level compared with what would be expected for a normal heterozygote (26% rather than 50%). This suggests the possibility of a mosaic for this mutation which has not been previously described in MPL. Summary. A novel homozygous nonsense mutation was identified in MPL by exome sequencing in a patient with familial severe aplastic anaemia. Screening of 33 uncharacterized AA patients revealed two additional novel mutations, one of which was recurrent and appeared to present as a mosaic. This study demonstrates that in a subgroup of patients with AA the disease is due to biallelic mutations in the MPL gene.

Table 1. Coding changes identified in MPL in AA.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age at sampling (diagnosis)</th>
<th>Coding change</th>
<th>Protein change</th>
<th>Exon</th>
<th>Status</th>
<th>Family segregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>3 years (3 years)</td>
<td>c.1248 G&gt;A</td>
<td>Trp416Stop</td>
<td>8</td>
<td>homozygous</td>
<td>yes</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>6 years (18 mo)</td>
<td>c.1180 C&gt;T</td>
<td>Pro394Ser</td>
<td>8</td>
<td>homozygous</td>
<td>yes</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>7 years (7 years)</td>
<td>c.1180 C&gt;T</td>
<td>Pro394Ser</td>
<td>8</td>
<td>heterozygous</td>
<td>n/a</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>7 years (7 years)</td>
<td>c.314 T&gt;A</td>
<td>Phe105Tyr</td>
<td>3</td>
<td>heterozygous</td>
<td>n/a</td>
</tr>
</tbody>
</table>

n/a = family samples unavailable.

Figure 1.

Figure 2.

Table 1. Coding changes identified in MPL in AA.
Hematopoiesis, stem cells and microenvironment

1030 COMBINING CELLULAR BARCODING AND MULTIPLE DEEP SEQUENCING FOR HIGH-RESOLUTION QUANTITATIVE CLONAL ANALYSIS IN THE HEMATOPOIETIC SYSTEM

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Background: Accurate assessment of clonality is important in a variety of hematopoietic stem cell research areas, including cell expansion, aging, gene therapy, cancer progression and treatment. However, detailed studies of stem cell clonality are hindered by the absence of methods with sufficient sensitivity and resolution to measure clonal contributions of both major and minor clones. Recently, we have designed and validated a novel cellular barcoding technique for clonal analysis of complex cell populations in vitro and in vivo (Gerrits et al., 2010). We have demonstrated that cellular barcoding combined with Sanger sequencing-based detection technique allows for detailed and unbiased assessment of clonal dynamics. Now, we coupled the barcoding approach with a high-throughput sequencing detection system and tested if such set-up will allow detailed quantitative analysis in heterogeneous populations of hematopoietic cells.

Methods and Results. First, we developed a multiplexing protocol which allows simultaneous analysis of up to 200 DNA samples containing barcoded cell populations in a single Solexa sequencing run. We applied this protocol to analyze clonal dynamics in cultures of primary mouse bone marrow cells transduced with barcoded vectors. A high number of sequence reads (4000 to 200000 reads per sample) allowed detailed and quantitative assessment of clonal fluctuations of over 100 barcodes in these cultures over time. Next, we transplanted barcoded murine bone marrow cells into irradiated recipients in a limiting dilution setting and followed the changes in barcodes pre- and post-transplantation. This allowed us to trace behavior of both individual stem cells in clonally-repopulated animals and analyze minor and major clones in polyclonal mice. Summary/Conclusions. These data confirm that cellular barcoding in combination with high-throughput sequencing is an effective tool for the study of cell population dynamics. This approach permits a quantitative, high-resolution assessment of clonality and offers an unprecedented sensitivity in the ability to analyze heterogeneous cell populations. In the future, we anticipate that this method can be used for detailed monitoring of clonal changes in gene therapy protocols.

1031 FORCED EXPRESSION OF THE HISTONE H3K36 DEMETHYLASE FBXL10/KDM2B MAINTAINS THE SELF-RENEWAL CAPACITY OF MOUSE HEMATOPOIETIC STEM CELLS

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Post-translational covalent modifications of histone N-terminal tails are central to the epigenetic regulation of transcription, replication, and repair. Histone methylation marks contribute to transcriptional activation or repression depending on the types and sites of modified residues and the degree of methylation. The methylation status of histones changes dramatically depending on cellular context and defines cell type-specific gene expression profiles. Histone demethylases have recently been implicated in this process. However, the role of the regulation of hematopoietic stem cells (HSCs) remain poorly understood. To explore the relevance of histone demethylases in HSCs, we profiled the expression of 26 histone demethylase genes in mouse hematopoietic cells and found that Fbxl10/kdm2b is highly expressed in Cd34+Kitt+Sca-1-Lineage marker (CD34KSL) HSCs and CD34KSL multipotent progenitors, but is markedly down-regulated during differentiation in bone marrow. Fbxl10 belongs to the jumonji C domain-containing histone demethylase family and is a demethylase specific to histone H3 mono/dimethylated at lysine 36 (H3K36me1/me2). Fbxl10 promotes the proliferation and functions as a physiological inhibitor of cell senescence in mouse embryonic fibroblasts through repression of p16/ink4a and p15/ink4b. Based on these data, we hypothesized that Fbxl10 plays a role in the maintenance of HSCs and investigated the role of Fbxl10 in HSCs by conducting a gain-of-function analysis. CD34KSL HSCs were transduced with an empty control or an Fbxl10 retrovirus and cultured in the presence of Flt3L and TPO. After 7 days, although Fbxl10-transduced CD34KSL HSCs gave no apparent growth advantage to cells, the percentage of KSL cells was 2-fold higher in the Fbxl10 culture than in the control culture. The total number of colony-forming cells (CFCs) derived from Fbxl10-transduced CD34KSL HSCs was slightly increased in the 3.5-day culture compared to the control, but increased up to 2-fold in the 10-day culture. Morphological evaluation of the colonies revealed that the control and Fbxl10 cultures contained comparable numbers of colony-forming unit-neutrophil/macrophage/Erythroblast/Megakaryocyte (CFU-nEM) with the potential for differentiation into multiple myeloid lineages at day 3.5 of culture while after 10-day culture, the Fbxl10 culture retained more CFU-nEM than the control culture. These data demonstrated that forced expression of Fbxl10 in CD34KSL HSCs expands CFCs with multi-lineage differentiation potential during ex vivo culture. Forced expression of Fbxl10 significantly repressed the expression of p16/ink4a, p15/ink4b, p18/ink4c, p19/arf and a moderate increase in H2Aub levels at these promoters and gene bodies on forced expression of Fbxl10 in Lineage- KSL cells. Competitive repopulation assays demonstrated that Fbxl10-transduced CD34KSL HSCs prevents exhaustion of the long-term repopulating potential of HSCs following serial transplantation. We conclude that the histone H3K36 demethylase, Fbxl10, is a novel epigenetic regulator in HSCs, which plays an important role in maintenance of self-renewal capacity and multipotency of HSCs.

1032 ESSENTIAL ROLE OF TIP49 IN HEMATOPOIETIC STEM CELLS

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Background. Tip48 and Tip49 are members of a conserved protein family with structural homology to bacterial ATP-dependent RuvB. In eukaryotes, Tip48 and Tip49 perform essential functions as RuvBs. In mammals, Tip48 is a histone H3K4 methyltransferase and Tip49 is a histone H3K36 demethylase. Tip49 regulates the function of several key regulators of cell proliferation, such as Myc, E2F, β-catenin, p53 and likely CDKs, as well as their function in mouse development and hematopoiesis. The role of Tip49 in hematopoietic system has not been yet investigated. Aims. The aim of this study is to evaluate the importance of Tip49 protein in vivo by conditionally deleting tip49 gene in mice. The role of Tip49 is addressed during mouse development as well as in adult hematopoietic tissues, specifically in hematopoietic stem cells (HSCs). Methods. A conditional allele of Tip49 gene in mice has been generated using a standard Cre-LoxP approach. The floxed gene was then bred into homozygocity (tip49 fl/fl). In order to generate tip49-null allele, mice carrying the conditional alleles were bred to a general Cre deleter line. To specifically delete Tip49 in adult mice, tip49 fl/fl mice were crossed to Mx1-Cre interferon-inducible line to efficiently delete tip49 in bone marrow, spleen and liver. Results. General deletion of Tip49 results in early embryonic lethality at periimplantation stage, while inducible deletion of tip49 in hematopoietic system also results in rapid lethality associated with anemia and pancytopenia. Bone marrow of tip49-deficient mice manifested a rapid drop in total cell number, associated with anemia and pancytopenia. Bone marrows of tip49-deficient mice manifested a rapid drop in total cell number, associated with anemia and pancytopenia. Bone marrows of tip49-deficient mice manifested a rapid drop in total cell number, associated with anemia and pancytopenia. The bone marrow of tip49-deficient mice also showed a decrease in granulocyte and monocyte progenitors, associated with a decrease in progenitor cells proliferation and disappearance of HSCs. The effects of tip49 gene deletion were hematopoietic cell autonomous, as Tip49 deletion in chimeric bone marrow led to an early apoptotic cell death exclusively in mutant long-term HSCs (Lin-Sca-1+CD150+) and not in the wild type competitor HSCs. Summary. These data demonstrate the essential role of transcriptional regulator Tip49 in adult hematopoietic stem cells, and suggests that modulation of its activity may represent a way to impinge on HSC functions.
Pleiotrophin is a secreted niche factor which regulates engraftment of hematopoietic stem cells

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Hematopoietic stem cells (HSC) are maintained at a quiescent state during steady-state hematopoiesis. HSC are characterised by their ability to respond quickly to hematopoietic stress in vivo, as well as in culture, by increasing proliferation and inducing early lineage commitment. It is likely that secreted niche factors are involved in this transition from quiescence towards activation. We here show that knockdown of the secreted factor pleiotrophin (Ptn-KD) in stromal cells increases proliferation as well as production of hematopoietic progenitors and HSC activity in co-cultures with lineage-negative (Lin-) hematopoietic cells. Moreover, engraftment of cells co-cultured with Ptn-KD stromal cells is associated with increased numbers of CD34-Lin-Sca1+ Kit+ (LSK) cells and dominant myeloid regeneration. Despite clear effects of Ptn deficiency in co-cultures, steady-state hematopoiesis is not altered in Ptn knockout (Ptn-/-) mice. This suggests that Ptn may be involved in limiting HSC activation, but is probably not involved in quiescence. Indeed, engraftment of wild-type HSC in lethally irradiated Ptn-/- mice mirrors the cultures on Ptn-KD stromal cells in that engraftment is increased in serial transplantsations with progressive myeloid skewing and accumulation of CD34-Lin-LSK donor cells. On a molecular level, steady state Ptn-/- LSK cells express decreased levels of cyclin D1, but an increased expression of the myeloid master regulator C/EBPalpha. In contrast, the observed increase in hematopoietic regeneration in co-cultures on Ptn-KD stromal cells is associated with URegulation of cyclin D1 (Ccdn1), whereas again C/EBPalpha was also increased. Thus, the difference between steady-state quiescence and activation of engraftment appears to lie in strict regulation of cyclin D1 through Ptn during early HSC activation. Interestingly, neither in steady state Ptn-/- cells, nor in wild-type LSK cells co-cultured on Ptn-KD stroma, did the regulation of cyclin D1 through Ptn depend on changes in transcript or protein levels of β-catenin. This finding indicates that regulation of HSC activation and engraftment through Ptn is independent of canonical Wnt signaling. In conclusion, our results support the hypothesis that Ptn secreted by the microenvironment is an important regulator of cyclin D1-dependent hematopoietic regeneration.

Energy metabolism of glucose and ATP affects the growth and differentiation of hematopoietic stem/progenitor cells

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Background and Aims. Recently, it has been reported that undifferentiated state of hematopoietic stem cells is regulated by their environmental factors, such as oxygen concentration. Furthermore, we have clarified that a NAD dependent histone deacetylase, Sirt1, is involved in the fate of hematopoietic stem cell as energy sensor. In this study, we examine the effects of energy metabolism on the proliferation and differentiation of hematopoietic stem/progenitor cells. Methods and Results. To clarify the relation of energy metabolism and cell cycle of hematopoietic stem/progenitor cells, murine bone marrow derived lineage(-), Sca1(+), c-Kit(high) (KSL) cells were double stained with Pyronin Y and Hoechst, and were divided into cells in G0, G1, and S/G2/M periods. The intracellular ATP concentrations rose as the cell cycles shift from G0, G1 to S/G2/M (0.25, 0.45, 0.60µg/cell, respectively). We also visualized intracellular NADH with 460nm excitation. Though NADH could be detected at a low level in cells in G0, potent fluorescence was observed in cells in S/G2/M. From these data, it was supposed that energy metabolism such as ATP or NADH production may be activated when a hematopoietic stem cell enters into cell cycle. Next, we cultured KSL cells in the medium containing 0, 50, 100, 150 or 200 mg/dL of glucose, supplemented with TPO, SCF, Flt-3L. Cell proliferations were promoted glucose-dose dependently. On the other hand, the residual KSL population was the highest in cells cultured with 50 mg/dL of glucose and the lowest in cells cultured with 200mg/dL. Next, we performed paired daughter cell colony assays. Murine bone marrow KSL cells were clonally sorted into 96 well plates and cultured with medium containing 50 or 200 mg/dL of glucose. When a sorted cell divided into two daughter cell pair, which were then separated by micromanipulation and transferred into methylcellulose medium containing 200µg/dL of glucose. We evaluated the period from sorting to the first division of each cell, and the colonies from each daughter cell pair were evaluated 8 days after manipulation. The periods of division were shorter in KSL cells cultured with 200µg/dL of glucose. If a stem cell is divided symmetrically into two stem cells, which means selfrenewal, the daughter cell pair will form mix/mix colony pair. Forty-four percent of daughter cell pairs from KSL cells cultured with 50mg/dL of glucose formed mix/mix colony pairs, in contrast to 22% in KSL cells cultured with 200mg/dL. These data demonstrated that low glucose concentration leads cell cycle suppression and promotes selfrenewal of hematopoietic stem/progenitor cells. We also performed the same paired daughter cell assays using KSL cells cultured with nicotinamide(NA), the inhibitor of Sirt1. NA-supplement cancelled completely the cell cycle suppression and partially mix/mix colony pair formation in KSL cells cultured in low dose glucose, suggesting that Sirt1 may be involved in the cell cycle suppression and the promotion of selfrenewal of hematopoietic stem/progenitor cells in low dose glucose environment. Conclusions. In hematopoietic stem/progenitor cells, the environmental glucose concentration and the intracellular energy metabolism are involved in the maintenance of stemness and the regulation of proliferation.
Granulocytes and signaling

Identification of a novel mode of kinase inhibitor resistance: An F604S exchange in FIP1L1-PDGFRA modulates FIP1L1-PDGFRA protein stability in a Src-2 and Src-dependent manner

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FIP1L1-PDGFR alpha is a constitutively activated protein kinase which is associated with chronic eosinophilic leukemia (CEL). Imatinib is clinically active in FIP1L1-PDGFR positive disease. However, clinical resistance to imatinib has been observed in FIP1L1-PDGFRA positive leukemia and was shown to occur due to secondary mutations in the PDGFR alpha kinase domain. Using a screening strategy to identify imatinib resistant mutations, we generated imatinib resistant single cell clones expressing FIP1L1-PDGFRA. Analysis of the PDGFR alpha kinase domain in these cell clones revealed a spectrum of resistance mutations including the clinically reported exchange T674I. Interestingly, one of the most frequent mutations was a Phe to Ser exchange at position 604 (F604S), which occurred alone or in combination with other exchanges. Surprisingly, FIP1L1-PDGFRα/F604S (F604S FP) did not increase the biochemical or cellular IC50 value to imatinib when compared to wild-type FIP1L1-PDGFRA (wt). However, F604S FP transformed Ba/F3, NIH3T3 and mouse bone marrow more efficiently compared to wt. Immunoprecipitation and immunoblotting indicated greatly increased amounts of F604S FP protein compared to wt in the cells. Pulse chase analysis revealed that F604S FP is strongly stabilized compared to wt. SRC coimmunoprecipitated with F604S FP but not with F604S FP. Co-expression of SRC in 293T cells augmented degradation of wt-FIP1L1-PDGFRA, but not F604S FP, indicating that SRC is a negative regulator of FIP1L1-PDGFRα protein stability. Accordingly, both the SRC inhibitor PD166326 and SRC siRNA mimicked the F604S phenotype and resulted in stabilization of the wt protein. Importantly, phosphatase inhibitor treatment of F604S FP led to destabilization and SRC recruitment indicating that phosphatases might be responsible for the enhanced stability of F604S FP. In fact, coimmunoprecipitation experiments identified the phosphatase SHP2 as a specific binding partner of F604S and mapping experiments revealed that the phosphatase domain of SHP2 directly interacted with F604S FP but not with wt- FIP1L1-PDGFRα. Together, these results suggest that stabilization of F604S FP is due to dephosphorylation by SHP2 leading to lower activation of the SRC and Cbl mediated ubiquitination machinery. Therefore this work identified a novel class of resistance mutations in FIP1L1-PDGFRA, that do not act by im peding drug binding to the target, but increase tumor protein stability and ability to interfere with SRC- mediated degradation.

Targeting the cdc2-C/EBPA pathway induces differentiation of human acute myeloid leukemias with FLT3ITD activating mutations

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Activation of mutations in FLT3 (fms-like tyrosine kinase 3) receptor, such as FLT3ITD, are among the most prevalent mutations in acute myeloid leukemias. The oncogenic role of FLT3 mutants has been attributed to the abnormal activation of several downstream signaling pathways, such as STAT5, STAT3, AKT, or ERK1/2. We have previously demonstrated that the kinase catalytic domain of FLT3ITD can phosphorylate C/EBPA on serine 21, and consequently inactivate its function. C/EBPA is a transcription factor playing a critical role in granulocytic differentiation and often inactivated in various subtypes of leukemia by multiple mechanisms. Among FLT3ITD patients, only 89% demonstrated activation of MEK1, and thus the ERK1/2 pathway, yet we show here that C/EBPA can be still phosphorylated on serine 21. We identified cdc2 (also known as CDK1) as a novel FLT3ITD activated kinase and we determined that FLT3ITD mutant receptors superactivate cdc2 via upregulation of cyclins A and B. Furthermore, we demonstrate that cdc2 directly phosphorylates C/EBPA on serine 21 (in vitro and in vivo), which inhibits its differentiation-inhibiting function. Importantly, we found that pharmacological and genetic (knock-down) inhibition of cdc2 activity relieves the differentiation block in FLT3ITD cell lines. Next, we investigated the effect of cdc2 inhibitor, NU6102, in primary FLT3ITD leukemia patient samples collected at diagnosis. As expected, cdc2 inhibition led to a remarkable hypophosphorylation of C/EBPA and a dose-dependent decrease in immunostaining of myeloid surface marker expression, such as CD15, as well as increase in CD15 expression. Moreover, the treatment of FLT3ITD carrying specimens with NU6102 for 7 days was accompanied by morphological changes suggesting granulocytic differentiation. Altogether, our data indicate that FLT3ITD mutants superactivate cdc2, which leads to phosphorylation of C/EBPA at serine 21 resulting in a differentiation block. Clinical trials with cdc2 inhibitors are currently under way for various malignancies. Our data strongly suggest that targeting the cdc2 pathway might be applied for the treatment of FLT3ITD mutant leukemias, especially of those resistant to FLT3 inhibitor therapies.

Comparative genome-scale RNA interference screens identify the MLL-fusion-associated gene AF4 as a regulator of CD133 transcription

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Background. The AC133 epitope expressed on the pentaspan transmembrane glycoprotein CD133 was first discovered as a cell-surface marker of hematopoietic progenitor cells. Since then, it has been used as a cancer stem cell marker in various human cancer cell lines. Although numerous studies have reported that DNA methylation of the CD133 promoter regulates its transcription regulation, the specific transcription factors involved remains poorly understood. Aim. To identify factors involved in regulating CD133 transcription. Methods. We performed a pooled short-hairpin RNA interference screen targeting >11,000 human genes in the CD133 endogenously expressing human epithelial colorectal adenocarcinoma Caco-2 cell line and in an engineered human embryonic kidney (HEK) 293 line exogenously expressing CD133. shRNA knockdowns that resulted in decreased cell-surface CD133 expression as determined by fluorescent-activated cell sorting were considered hits and their identities were deconvoluted using custom microarrays. To identify genes involved in endogenous CD133 transcription, we focused on shRNA hits specifically required for CD133 expression in Caco-2 cells, but not in the HEK 293/CD133 line. Results. We identified the transcription activator AF4 as a regulatory of CD133 transcription, as gene knockdown of AF4 results in a dramatic reduction in CD133 transcript levels. It has been well established that AF4 is associated as a MLL-fusion in acute lymphoblastic leukemia (ALL). Consistent with our findings, MLL-AF4 has been demonstrated to interact with the CD133 promoter in the ALL cell line SEM. Furthermore, MLL-AF4/AF4 has shown to be involved in a protein complex involved in transcription elongation. When we performed gene targeted knockdown of complex members, we observed a significant decrease in CD133 transcript, suggesting that MLL-AF4 and AF4 functions in this protein complex to regulate CD133 transcription elongation. Summary. Our study provides mechanistic insight into the transcriptional regulation of CD133 in MLL-AF4 expressing ALL cell lines and in non-leukemia cell lines.

Differential stimulation of CEBPA target genes during ATRA-induced granulocytosis versus CDDO-induced granulomonocytic differentiation

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Background. Induction of differentiation may be a powerful means to treat human acute myeloid leukemia (AML). However, while all-trans retinoic acid (ATRA), which induces granulocytic differentiation, has been applied successfully in patients with acute promyelocytic leukemia, the clinical benefit in the other AML subgroups of patients has been much less promising. Aim. We have previously shown that 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO) induces granulomonocytic differentiation, and this was at least in part mediated by transcriptional activation of the transcription factor CCAAT Enhancer Binding
Protein alpha (CEBPA) (Koschmieder et al., Blood 2007). Here, we investigated similarities and differences between ATRA- and CDDO-induced effects. Methods. HL60, 32D, or 293T cells were used for analysis of RNA expression by RT-PCR, CEBPA transactivation potential using luciferase assays, and CEBPA target identification by chromatin immunoprecipitation (ChIP)-chip array techniques. Results. Both ATRA (1 μM) and CDDO (0.5 μM) increased Id2 mRNA in HL60 cells, although Id1 induction was less pronounced with CDDO. Since Id1 and Id2 are CEBPA target genes, we analyzed CEBPA binding to Id1 and Id2 target genes and found that CEBPA bound to Id1 and Id2 promoters as well as the Id1 enhancer. CEBPA stimulated luciferase activity of the Id1 enhancer but not the Id1 promoter. CEBPA, CEBPB, and CEBFD stimulated Id1 enhancer activity to different extents. While ATRA and CDDO only weakly stimulated Id1 enhancer activity in parental 32D cells, concomitant transfection of CEBPA, CEBPB, or CEBFD additively increased this activity. ChIP-Chip array analysis of CEBPA binding in HL60 cells demonstrated remarkable similarities of known and novel CEBPA target genes induced to different extents. Moreover, while ATRA and CDDO only weakly stimulated luciferase activity of the Id1 enhancer but not the Id1 promoter, CEBPA bound to Id1 and Id2 promoters as well as the Id1 enhancer. CEBPA stimulated luciferase activity of the Id1 enhancer but not the Id1 promoter. CEBPA, CEBPB, and CEBFD stimulated Id1 enhancer activity to different extents. While ATRA and CDDO only weakly stimulated Id1 enhancer activity in parental 32D cells, concomitant transfection of CEBPA, CEBPB, or CEBFD additively increased this activity. ChIP-Chip array analysis of CEBPA binding in HL60 cells demonstrated remarkable similarities of known and novel CEBPA target genes induced by both ATRA and CDDO, including increased binding to Myd88, Vva1, IIL-10, Gpr2, Cas2, ILS and decreased binding to Cach1 and EpOr. Conclusions. The differentiation inducers ATRA and CDDO stimulate similar pathways of granulocytic differentiation involving CEBPA and its target genes. In addition, our ChIP-chip array approach identified novel targets of CEBPA which may enhance our understanding of cell fate decisions during granulocytic versus monocytic differentiation.

1039 FAS GENE EXPRESSION IS EPIGENETICALLY REGULATED AND PREDICTS THE RESPONSIVENESS TO AZATIDINE IN HIGH-RISK MYELODYSPLASTIC SYNDROMES

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Background. Low risk myelodysplastic syndromes (MDS) CD34+ cells exhibit high level of the death receptor Fas at their surface and abnormal Fas-dependent apoptosis. Fas expression decreases when the disease progresses to acute myeloid leukemia (AML). Based on recent evidence of higher Fas level in high-risk MDS, we explored the epigenetic regulation of FAS gene during MDS evolution to AML. Aims. This study aims at investigating the regulation of Fas in FasL in MDS/AML cells. Methods. We quantify Fas gene expression by RT-qPCR in bone marrow mononuclear cells (BM/MNC) from 156 patients (86 MDS, 30 AML) and in 20 controls, including 6 patients treated with azacitidine according to the FDA/EMEA schedule. FAS expression is scored according to the H3K4me2, H3K9me2, H3K27me3) are performed in HL60 cell line, according to the FDA/EMEA schedule. Response is scored according to the IPSS, age and previous treatment. Results. Among patients transplanted in CR1 within intermediate risk group, 2 y LFS was 56% advanced, p=0.001). Relapse incidence was 31% for those patients transplanted with MAC (n=291) and 30% with RIC (n=282). The 2 y probability of leukemia-free-survival (LFS) was 33±2% for MAC, 41±2% CR2 or CR3, 56±advanced, p<0.01). In patients given a MAC, 2y LFS was 50% for CR1, 27% for CR2 or CR3 and 17% for more advanced phase of the disease whereas it was 35%, 44% and 15% for LFS respectively. Among patients transplanted in CR1 within intermediate risk group, 2 y LFS was 46±6% and it was 33±8% for those within unfavorable risk group, while for those transplanted in CR2 it was 38±6% and 30±9%, respectively. In multivariate analysis, disease status at transplant (remission vs advanced) was the main factor associated with improved LFS (HR 2.12, 95% CI 1.3-3.15, p<0.01). Causes of death were infections or other transplant-related events (n=195) or disease progression (n=149). In conclusion, this large series of patients shows that UCBT is an option treatment for adults with high risk AML after a myeloablative or reduced conditioning regimen without a suitable HLA matched donor.

1040 SINGLE AND DOUBLE CORD BLOOD TRANSPLANTATION FOR ADULT WITH ACUTE MYELOID LEUKAEMIA: A SURVEY ON BEHALF OF EUROCORD AND ALWP-EBMT

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Background. Patients with high-risk acute myeloid leukemia (AML) have few chances of cure without allogeneic stem cell transplantation (HSCT). HSCT can be used in first remission for pts with poor-risk cytogenetics, as rescue for pts refractory to chemotherapy, at first relapse or in secondary and subsequent remission. UCB is an established stem cell source for HSCT. Methods. We retrospectively analyzed 604 adult (≥18y) with de novo AML who received UCBT as first transplant. Results. 292 patients were transplanted in first complete remission (CR1, 228 in second or third CR and 147 in advanced disease. Patients were transplanted from 2000-2010 in 131 EBMT centers. Median age was 41 years, 18% of the patients received a previous autologous transplantation. Based on available cytogenetic and molecular markers at diagnosis (n=539) 56% were in intermediate risk and 31% in unfavorable risk group. Grafts were composed of 1 (sUCBT) (n=361) or 2 (dUCBT) (n=243) CB units, 39% of CB units were identical to recipient or had 1 HLA disparity (antigen level for HLA-A and B allele level for DRB1) while 61% had 2-3 HLA disparities. At infusion median TNC cell dose was 3.1±10^7/kg (2.4±10^7/kg with sUCBT and 3.7±10^7/kg with dUCBT) and median CD34+, 1.2±10^5/kg (1x10^5/kg with sUCBT and 1.5±10^5/kg with dUCBT). Fifty-one percent of pts received a myeloblastic conditioning regimen (MAC) and 49% a reduced intensity regimen (RIC). The most common regimens used were busulphan-fludarabine-thiotepa for MAC and cyclophosphamide+fludarabine+TBI2Gy for RIC. GVHD prophylaxis consisted of CSA+MMF in 58% of pts and CSA+steroids in 42%. Median follow-up was 18 months and it was 23 months for sUCBT and 15 months for dUCBT. Cumulative incidence (CI) of neutrophil recovery, acute GVHD (I-IV) and 1y TRM was 80±2%, 26±3% and 21±3%, respectively. CI of 2y relapse was 38±3% (27%, CR1, 29% CR2 and CR3, 56% advanced, p=0.001). Relapse incidence was 31% for those patients transplanted with MAC (n=291) and 30% with RIC (n=282). The 2y probability of leukemia-free-survival (LFS) was 33±2% (45%, CR1, 41% CR2, 16% advanced, p<0.01). In patients given a MAC, 2y LFS was 50% for CR1, 27% for CR2 or CR3 and 17% for more advanced phase of the disease whereas it was 35%, 44% and 15% for LFS respectively. Among patients transplanted in CR1 within intermediate risk group, 2 y LFS was 46±6% and it was 33±8% for those within unfavorable risk group, while for those transplanted in CR2 it was 38±6% and 30±9%, respectively. In multivariate analysis, disease status at transplant (remission vs advanced) was the main factor associated with improved LFS (HR 2.12, 95% CI 1.3-3.15, p<0.01). Causes of death were infections or other transplant-related events (n=195) or disease progression (n=149). Conclusion. In conclusion, this large series of patients shows that UCBT is an option treatment for adults with high risk AML after a myeloblastic or reduced conditioning regimen without a suitable HLA matched donor.

1041 COMBINED MISSING SELF MODEL AND LIGAND-LIGAND MODEL CAN PREDICT HIGHER RELAPSE RATE AFTER HLA-MISMATCHED TRANSPLANTATION WITHOUT T CELLS DEPLETION IN VITRO

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Background. HLA-mismatched/haploidentical stem cell transplantation (SCT) is a feasible therapeutic option for advanced hematologic malignancies. In some of patients with HLA-mismatched SCT, especially in the context of the scarcity of bone marrow transplantation (BMT), the mismatching of HLA-A and -B and/or lack of DRB1 between donor and recipient is not uncommon. The recipients of HLA-mismatched SCT are at higher risk of severe acute and chronic GVHD due to the lack of missing self recognition, which results in the up-regulation of FasL expression driven by CEBPA. In our previous study, we found that Fas overexpression might enhance the risk of acute GVHD of the recipients. In contrast, we also observed that Fas underexpression might result in more tolerance of the recipients. The balance between the expression of Fas and its ligand FasL might be a key factor affecting the outcome of HLA-mismatched SCT. In this study, we applied combined missing self model and ligand-ligand model to predict the relapse rate of patients who received HLA-mismatched SCT without T cells depletion in vitro.
logic malignancies patients who lack an HLA-matched related or unrelated donor. Conflicting results have been reported about the impact of alloreactivity of natural killer (NK) cells on the outcome of haploidentical SCT to leukemic patients. Aims. The goal of this study was to explore the predictive roles of missing self model in our HLA-mismatched/haploidentical transplantation without T-cell depletion in vivo, and to develop a simple algorithm on the basis of recipients and donor HLA-C and HLA-Bw4 gene content that can be used today to identify HLA-mismatched donors who will provide the most protection against relapse in T cell-replete transplants. Methods. We studied the HLA genotype of 153 donor-recipient pairs, who underwent unmanipulated HLA-mismatched/haploidentical transplantation without T-cell depletion in vivo. To apply the missing ligand model, the first step was to divide our donor-recipient pairs into 2 groups according to the number of KIR ligand in donor and recipient, ie, 3 KIR ligands (“without missing self”) versus fewer than 3 (“with missing self”). Meanwhile, to apply the KIR ligand-ligand model, donors who were classified as NK alloreactive against their recipients termed KIR ligand mismatched donors throughout, possessed HLA class I KIR ligands which were missing in the recipients. Results. Among the 153 pairs of donor-recipients, 110 and 43 recipients received HLA-mismatched/haploidentical transplants from “with missing self” and “without missing self” donors, respectively. Using Ligand-ligand model, 119 and 34 recipients received haploidential transplantation from “KIR ligand matched” and “KIR ligand mismatched” donors, respectively. In contrast to Perugia’s KIR ligand-ligand mismatched model or Handgretinger’s KIR missing self model between donor-recipient pairs, we found that the cumulative incidence of 7-year relapse rate were higher in patients received transplantation from “with missing self” or “with KIR ligand mismatched” donors compared with those from “without missing self” (p=0.00746) or “without KIR ligand mismatched” (p=0.01194) donors, respectively. When combined the above predictive model together, patients were subgrouped as receiving graft from “without missing self and without KIR ligand mismatch” (best, n=45), “with missing self and without KIR ligand mismatch” (better, n=76), and “with missing self and with KIR ligand mismatch” (neutral, n=54), respectively. We found the 7-year disease-free survival (DFS), overall survival (OS), and relapse rate were best predicted by the combination of missing self and KIR ligand mismatch between recipients and donors pairs (HR 1.517(1.081-2.128), p=0.016 for DFS; HR 1.518(1.081-2.142), P=0.016 for OS; HR 2.46(1.424-4.205), P=0.001 for relapse, figure1).

Conclusions. These data indicate that poor prognosis after transplantation is associated with the missing self and KIR ligand mismatch in recipients and T cell alloreactive role may play a predominant role in this model. Meanwhile, we developed a simple algorithm on the basis of recipients and donor HLA-C and HLA-Bw4 gene content that can be used today to identify HLA-mismatched donors who will provide the most protection against relapse in T cell-replete transplants.
the standard of care: 104 (41%) patients found an UD or UCB unit after a median time of 1.6 months (0.3-26); 86 with UD of which only 60 with UCB of which only 17 were transplanted. Among transplanted patients, 113 (74%) were in CR, 40 in <CR. Fifty (33%) received PBSC, with UCB 14 HLA 4/6 and 3 HLA 5/6. Re-

sults. After a median follow-up of 25 months (0.2- 234), the median OS was 78 months (51-135) for transplanted patients with SD (3years OS: 68%); it was 33 months (27-47) for transplanted patients with UD (3years OS:44%); 21 months (15-37) for not transplanted patients with available SD or UD (5years OS:34%) and it was 51 months (23-221) for patients with ND (3years OS:45%). Median EFS for the same groups was 38 months (23-133), 24 months (17-36), 15 months (11-24) and 23 months (14-48) respectively. In multivariate analysis, 8 significant fac-
tors affected OS: disease status (<CR) HR= 2.8 [1.5-5.3] p=0.001; long interval diagnosis-registration HR= 2 [1.2-3.6] p=0.001 and condi-
tioning (standard) HR=0.27 [0.1-0.8] p=0.02. Conclusion. The interval di-
agnosis-registration appeared as major factor affecting survival in UD allo-HSCT settings.

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UNRELATED CORD BLOOD TRANSPLANTATION (UCBT) FOR CHILDREN WITH ACUTE MYELOID LEUKAEMIA (AML): AN ANALYSIS OF EUROCORD, ALWP AND PDWP OF EBMT

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Background. In 2003, Eurocord group has described outcomes after UCBT in 95 children with AML. Aims. Better define the role and to identify prognostic factors of UCBT in childhood with AML. Methods. Retrospective analyses of 390 children with AML who received single UCBT in EBMT centres. Results. Median age and median weight at transplantation were performed from 1994 to 2010: 57% during CR1, 42% in CR2 and 21% in more ad-

vanced disease. On the basis of cytogenetic and molecular characteris-
tics, 253 children (65%) were stratified into 3 groups: 35% in the un-
favourable, 22% in the intermediate and 8% in the favourable. The majority of grafts were HLA 5/6 (44%) or 4/6 (37%). Median number of infused total nucleated (TNC) and CD34+ cells was 4.9x10^7/kg and 1.9x10^6/kg, respectively. The majority of patients (86%) received myeloablative conditioning regimen with TBI (25%) or busulfan (60%). ATG was used in 80% of cases. Median follow-up time was 24 months. Median time to achieve neutrophil and platelet recoveries was 24 and 42 days. Cumulative incidence (CI) of ANC recovery was 85%; in a multivariate model it was favourably associated with a higher TNC dose (> median, HR: 1.40, p=0.008) and transplantation in CR1 (HR: 1.39, p=0.015). At day 100, CI of grade II-IV acute GvHD was 34% (11% grade III, 5% grade IV). At 2y CI of NRM was 24%. Mul-
tivariate analysis showed that: TNC dose (HR: 0.58, p=.024) and dis-
ease status (CR1 vs others) at time of UCBT (HR: 0.55, p=.026) were associated with decreased NRM. There was a trend toward a de-
creased NRM in patients given a 6/6 or 5/6 HLA graft (p=.06). CI of re-
lapse at 2 years was 17% in CR1, 26% in CR2 and 44% for more ad-

vance disease. Estimated 2 y-LFS was 63% in CR1, 43% in CR2 and 22% in more advanced patients. LFS of 49 children transplanted in CR1 with unfavourable disease was 70±7 %, not statistically different from the overall CR1 group. For those transplanted in CR2, 2y-LFS was 71±9%, 33±9 % and 40±7% in the favourable, intermediate and unfavorable subgroup. Multivariate analysis in CR2 cohort identified 2 significant prognostic factors: favorable disease (HR,3.74, p=.005) and previous CR1 duration longer than 7 months (the threshold of the first quartile, HR,1.85, p=.03). Conclusions. We conclude that UCB is an attractive stem cell source in childhood AML when no HLA-identical donor is available. Cell dose remains an important factor for engraft-
ment and NRM. Results are very encouraging for unfavorable diseases in CR1.

Figure 1.
Progress in the treatment of non-Hodgkin Lymphomas

1045
DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) IN VERY ELDERLY PATIENTS (OLDER THAN 80 YEARS): PROMISING RESULTS IN THE ERA OF CHEMOIMMUNOTHERAPY DESPITE LOWER DOSE INTENSITY

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Background. The optimal treatment of very elderly patients with DLBCL has not been well established. Many patients have received chemotherapy (CIT) without anthracyclines with rather poor results. The combination of Rituximab with CHOP has greatly improved the prognosis of DLBCL. The administration of Rituximab in very elderly patients might permit dose reductions in the chemotherapy regimen without significant loss in efficacy. Aims. To describe the clinical characteristics, the actually delivered doses of chemotherapy and immunotherapy (CIT) drugs in routine clinical practice and the final outcome of treated patients with DLBCL, in the era of CIT. Patients and Methods. Among 579 patients with DLBCL treated in 5 medical Centers in the era of CIT, 56 were older than 80 years (10%). We studied the clinical and laboratory characteristics of these patients (individual factors of IPI, gender, B-symptoms, anemia, lymphocytopenia, albumin), Progression Free Survival (PFS) and Overall Survival (OS) in comparison with 523 patients younger than 60 years, who were treated during the same period. In addition, we analyzed the relative dose intensity (RDI) of the 4 drugs (except of corticosteroids), which was actually delivered to the very elderly patients, using as reference doses and time intervals of standard R-CHOP-21. Results. The median age of the very elderly patients was 82.5 years (80-91). The frequency of adverse prognostic factors (IPI, R-IPI, low serum albumin, severe lymphocytopenia) was significantly higher in very elderly patients, but the difference was mainly due to the favorable prognostic profile of the younger patient group (<60 years). In contrast, the characteristics of patients aged 61-79 and ≥80 years were similar. The 5-year PFS was 81%, 68% and 62% (p=0.0013) in the group of ≤60, 61-79 and ≥80 year-old patients respectively (p=0.12 for 61-79 vs. ≥80). The corresponding 5-year OS was 90%, 65% and 55% (p=0.0011, but p=0.048 for 61-79 vs. ≥80). In multivariate analysis of PFS, age ≥80 years had no independent prognostic significance when IPI and lymphocytopenia were taken into account. On the contrary, it was an independent prognostic factor for OS with a relative risk 2.9 (p<0.001) and 2.1 (p=0.01) compared to patients <80 years or 61-79 years respectively. Among patients ≥80 years-old, who received >1 cycle of anthracycline-based CIT, the median RDI and IQR were: Rituximab 85% (78-99), cyclophosphamide 76% (67-86), anthracycline 60% (50-69), vincristine 64% (50-76). Moreover, 6/56 patients did not receive anthracyclines and 2 received only 1 cycle. Summary/Conclusions. CIT provides prolonged survival in >50% of DLBCL patients ≥80 years-old. These results are very encouraging even though RDI for chemotherapy drugs was low in the everyday clinical practice. Age ≥80 years was not an independent prognostic factor for PFS but only for OS. These observations suggest that the majority of very elderly patients with DLBCL should not be treated with palliative approaches. Instead they should receive as complete R-CHOP-like CIT as possible with curative intent.

TREATMENT OF SPLENIC MARGINAL ZONE LYMPHOMA WITH RITUXIMAB MONOTHERAPY VS SPLENECTOMY

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Background. Treatment of splenomarginal zone lymphomas (SMZL) is not standardized. Splenectomy has traditionally been considered as the treatment of choice. Recent data indicate that rituximab is highly effective and that it could be considered as initial therapy for SMZL. Aims. To compare the efficacy of rituximab monotherapy versus splenectomy in SMZL patients. Methods. The studied population included 57 patients with SMZL who were diagnosed and prospectively treated in our Departments between September 2003 and July 2010 with rituximab monotherapy (induction phase at a dose of 375 mg/m² per week for 6 weeks and maintenance phase every 2 months for one year) and 25 patients who were diagnosed prior to rituximab period and who were faced by splenectomy only. Rituximab treated patients were evaluated for response 2 months after induction and 2 months after the end of maintenance phase. Demographic features, clinical and laboratory characteristics, type of response, response duration, overall survival, progression-free survival and cause of death were analyzed in all patients. Results. Patients characteristics were similar between the two treatment groups. The overall response rate (ORR) to rituximab after the end of induction phase was 92% (46% CR, 19% CRu, 13% PR, 13% CHR). The median time to hematologic and clinical response was 4 and 5 weeks respectively. 31/52 patients have already completed the maintenance phase, 29 of them (81%) sustained their initial response, while 5 improved their response and one progressed. 85% of splenectomized patients achieved clinical and haematological remission. The 5-year OS and PFS for rituximab treated and splenectomised patients was 97±5% and 75±9% (p value=0.01) respectively and 58±10% and 56±11% (p value=0.49) respectively (Table 1). Rituximab seems to confer a survival advantage over splenectomy, although so far no difference was noticed in the 5-year PFS time. One toxic death was recorded in the splenectomy arm. In the rituximab arm grade 2 neutropenia was noticed in one patient, grade 3 thrombocytopenia in another, while one patient could not complete therapy due to severe hypotension. Conclusions. Rituximab is a highly effective and well tolerated therapy and it can substitute splenectomy as the treatment of choice for SMZL.

Table 1. Rituximab vs splenectomy in SMZL pts.

<table>
<thead>
<tr>
<th>Rituximab</th>
<th>Pts (%)</th>
<th>ORR (%)</th>
<th>CR (%)</th>
<th>PR (%)</th>
<th>CHR (%)</th>
<th>5-year OS (%)</th>
<th>5-year PFS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>52</td>
<td>92</td>
<td>46</td>
<td>19</td>
<td>13</td>
<td>75±9</td>
<td>97±5</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>32</td>
<td>85</td>
<td>46</td>
<td>19</td>
<td>13</td>
<td>75±9</td>
<td>97±5</td>
</tr>
</tbody>
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TREATMENT OF SPLENIC MARGINAL ZONE LYMPHOMA WITH RITUXIMAB MONOTHERAPY VS SPLENECTOMY

Aims. To compare the efficacy of rituximab monotherapy versus splenectomy in SMZL patients. Methods. The studied population included 57 patients with SMZL who were diagnosed and prospectively treated in our Departments between September 2003 and July 2010 with rituximab monotherapy (induction phase at a dose of 375 mg/m² per week for 6 weeks and maintenance phase every 2 months for one year) and 25 patients who were diagnosed prior to rituximab period and who were faced by splenectomy only. Rituximab treated patients were evaluated for response 2 months after induction and 2 months after the end of maintenance phase. Demographic features, clinical and laboratory characteristics, type of response, response duration, overall survival, progression-free survival and cause of death were analyzed in all patients. Results. Patients characteristics were similar between the two treatment groups. The overall response rate (ORR) to rituximab after the end of induction phase was 92% (46% CR, 19% CRu, 13% PR, 13% CHR). The median time to hematologic and clinical response was 4 and 5 weeks respectively. 31/52 patients have already completed the maintenance phase, 29 of them (81%) sustained their initial response, while 5 improved their response and one progressed. 85% of splenectomized patients achieved clinical and haematological remission. The 5-year OS and PFS for rituximab treated and splenectomised patients was 97±5% and 75±9% (p value=0.01) respectively and 58±10% and 56±11% (p value=0.49) respectively (Table 1). Rituximab seems to confer a survival advantage over splenectomy, although so far no difference was noticed in the 5-year PFS time. One toxic death was recorded in the splenectomy arm. In the rituximab arm grade 2 neutropenia was noticed in one patient, grade 3 thrombocytopenia in another, while one patient could not complete therapy due to severe hypotension. Conclusions. Rituximab is a highly effective and well tolerated therapy and it can substitute splenectomy as the treatment of choice for SMZL.
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INFLUENCE OF THE TYPE OF IMMUNOSUPPRESSIVE THERAPY IN THE DEVELOPMENT OF PTLD FOLLOWING ORTHOTOPIC LIVER TRANSPLANT: A SINGLE CENTER, LONG-TERM SURVEY ON 1,649 PATIENTS
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Background. Post-transplant lymphoproliferative disorder (PTLD) is a serious complication in solid organ transplant recipients. The incidence is quite variable, and the role of the immunosuppressive treatments on the risk of PTLD development remains to be elucidated. Aims. To evaluate the frequency and the risk factors of PTLD in a large series of Orthotopic Liver Transplant (OLT). Patients and Methods. Data have been collected on 1,605 OLT performed in 1,649 patients at the Liver Transplant Center in Torino, Italy, during the period 1990 - 2008. OLT was performed in patients aged up to 65 yr, old, with 60 pediatric patients (age at OLT < 12 ys) and two patients aged 65 to 68 yr. The most common indications for OLT were viral cirrhosis, biliary disease and alcoholic cirrhosis. Cyclosporine A (CsA) has been used as primary immunosuppression in most patients; in the last few years, tacrolimus has been increasingly employed as primary immunosuppressive drug; steroids are usually associated to Calcineurin-inhibitors. Overall, 1,189 (73.8%) patients received CsA in prevalence, 423 (26.2%) had tacrolimus in prevalence. PTLD were diagnosed by histology and immunohistopathological analysis on biopsy specimens. Several parameters were evaluated for possible association with PTLD occurrence, including age, sex, liver disease and HCV state, presence of hepatocellular carcinoma, time elapsed from OLT to PTLD, CsA vs tacrolimus use, other drugs for graft rejection. The cumulative incidence of PTLD was determined using the Fine and Gray competing risk regression model. Treatment for PTLD included: i. reduction or discontinuation of the immunosuppression; ii. chemotherapy and/or radiotherapy (15 cases); Rituximab was delivered to 11 patients (combined with chemotherapy in 6). Results. At a median follow-up of 5.8 yrs. (range 0.1-17.5), 1,298 (78.7%) patients are alive, with a 5-yr Overall Survival projection of 79.5%. So far, 20 PTLD have been recorded, with a cumulative incidence of 0.94, 1.57 and 3.03% at 5, 10 and 15 yrs, respectively. Median time of PTLD occurrence was 32 mos. (range 2-155) since OLT. On competing risk multivariate analysis, the use of tacrolimus vs. CsA was the main factor associated with increased risk of PTLD (SDHR: 2.54, p=0.041). Despite the increased PTLD incidence, the overall risk of death was significantly lower with tacrolimus compared to CsA. An increased risk of PTLD was also observed in the 83 patients receiving OKT3, with a SDHR of 3.77, p=0.013. None of the other parameters had any significant impact on PTLD development. Treatment of PTLD resulted in good response in most patients and at a median follow up of 4.85 yrs., 17 out of 20 patients (85%) are alive. Conclusions. The overall incidence of PTLD in this large series of OLT is among the lowest reported so far in patients receiving solid organ transplant; the use of tacrolimus is shown as a significant risk factor for PTLD; nevertheless, the addition of tacrolimus significantly increased the life expectancy following OLT; the study confirms the improved outcome of PTLD and the availability of Rituximab is quite likely to have contributed to the prolonged survival observed.

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THE USE OF INTERIM 18FDG-PET IS NOT JUSTIFIED IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) DURING FIRST LINE IMMUNOCHEMOTHERAPY
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Background. In diffuse-large-B-cell-lymphoma (DLBCL), the response to first line immuno-chemotherapy remains somewhat unpredictable. Interim 18FDG-PET (PET-int) analysis could fulfill this important goal, aiding early shift to intensified regimens. Methods. We prospectively evaluated the ability of PET-int carried out at mid-treatment of standard immuno-chemotherapy in predicting relapse in a series of 83 consecutive DLBCL. PET-int results were dichotomized as positive or negative using the recently validated five-point-scale scoring system. This exam was also compared with interim computerized tomography (CT-int) and final PET (PET-fin). End-points were: complete remission (CR); positive predictive value (PPV) of refractoriness and relapse; negative predictive value (NPV); overall survival (OS) and progression free survival (PFS). Observation time was fixed to 24 months unless a DLBCL-related event. Results. The PPV of PET-int was 63.6% and the NPV was 81.9%. Within PET-int positive patients who underwent targeted biopsy the incidence of faulty positive results due to inflammation was 57%. The achievement of CR was correlated with both PET-int and CT-int (p=0.0001), but in multivariate analysis only CT-int was correlated with CR (p=.0002). CT-int and PET-fin were predictive of both OS and PFS, whereas PET-int was predictive only of OS (p=.018), but not of PFS (p=.105). In Cox-regression only PET-fin was predictive for both OS (p=.002) and PFS (p=.006). Conclusions. PET-int resulted unable to discriminate chemo sensitive patients who will later relapse. Therefore we think that the use of this expensive and radioactive tool is not justified as an interim analysis.

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CLINICAL SIGNIFICANCE OF METABOLIC TUMOR VOLUME BY 18F-FDG PET IN THE STAGE II AND III OF DIFFUSE LARGE B CELL LYMPHOMA
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Background. Aggressive Non Hodgkin’s Lymphoma (NHL) has been staged according to the Ann-Arbor staging system, which originally designed for Hodgkin lymphoma (HL). Because of the heterogeneity and hematogenous spread pattern of dissemination in NHL in contrast to contiguous lymphatic spread with HL, Ann-Arbor staging system has a limited value in the context of assessment of accurate tumor burden in NHL. Aims. The objective of the present study is to investigate whether metabolic tumor volume (MTV) by PET can be a potential prognostic tool compared with Ann-Arbor stage in the patient with stage II and III nodal diffuse large B cell lymphoma (DLBCL). Methods. One hundred sixty patients with de novo nodal DLBCL whom underwent PET-CT at diagnosis were enrolled for the present study. All patients received 6 to 8 cycles of R-CHOP therapy by Coiffier et al. Median follow-up duration was 36 months. MTV was delineated on the PET images by a circle encompassing regions equal or greater than standard uptake volume (SUV) 2.5 in involved lymph Node (LN) and each slice was determined by multiplying the area within the threshold margin by CT interval. The final MTV was calculated by adding all MTA of each slice. Results. The differences of several prognostic factors between stage II and III
groups were not present. ROC curve analysis was used to calculate the accuracy of ideal cut-off value to distinguish between low MTV and high MTV group. Various cut-off values of MTV were used to obtain a reasonable balance of sensitivity and specificity. 169.8 cm³ of various values acquired a sensitivity of 91.7% and specificity of 65.3%. The outcomes were compared among the four subgroups based on tumor burden and stage II or III. High MTV group regardless of stage had lower PFS and OS pattern compared to low MTV group (PFS & OS in stage II with low MTV, 95.2% & 96.8%; in stage III with low MTV, 90.5% & 90.5% versus in stage II with high MTV, 60.6% & 60.6%; in stage III with high MTV 48.8% & 48.8%; p<0.001, p<0.001) whereas prognostic impact of stage in same MTV group was absent. (in the low MTV group, difference of PFS and OS according to stage, p=0.481, p=0.261; in the high MTV, p=0.277, p=0.28, Figure 1). Multivariate analysis using Cox proportional hazard model was performed for high MTV and stage III. In the analysis high MTV was an independent factor predicting an unfavorable outcome (PFS, HR=9.245, 95% CI=3.543-24.115, p<0.001; OS, HR=11.660, 95% CI=4.062-34.465, p<0.001) whereas stage III had not significant value (PFS, HR=1.513, 95% CI=0.792-2.888, p=0.210; OS, HR=1.556, 95% CI=0.806-3.003, P=0.188). Conclusion. The present study suggests that total tumor burden of lymphoma is a more important prognostic parameter rather than Ann-Arbor stage in DLBCL. Our data reflect that Ann-Arbor staging system has limited worth in DLBCL due to heterogenous spread pattern of NHL. Therefore, simply classifying for prognosis according to diaphragm would be not wise at least for DLBCL in mtximab era.

**Figure 1. Comparisons of survival according to MTV and stage.**

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**MISSENSE MUTATIONS LOCATED IN STRUCTURAL P53 DNA-BINDING MOTIFS ARE ASSOCIATED WITH EXTREMELY POOR SURVIVAL IN CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background.** There is a distinct connection between TP53 defects and poor prognosis in chronic lymphocytic leukemia (CLL). It remains unclear whether patients harboring TP53 mutations represent a homogeneous prognostic group. p53 missense mutations involved in a direct or indirect contact with the target DNA, have been shown to be associated with a particularly poor survival in, e.g., breast cancer or diffuse large B-cell lymphoma. Aims. To analyze an impact of specific p53 mutations on prognosis of CLL patients. Methods. We evaluated the survival of CLL patients with p53 defects identified at our institution by p53 yeast functional assay (FASAY) and complementary I-FISH analysis detecting del(17p) from 2003-2010. Results. A defect of the TP53 gene was identified in 100 of 550 patients. p53 mutations (n=96 patients) were strongly associated with the deletion of 17p and the unmutated IgVH locus (both P<0.001). The patients who had p53 mutation but also the mutated IgVH gene (range of homology 92.4%-97.9%) manifested substantially better survival than p53-mutated patients with the unmutated IgVH (homology ≥98%) (P=0.018). We therefore omitted the small subgroup (n=11) of p53-affected patients with mutated IgVH gene from the subsequent analysis, as their survival data would be misleading. In line with this, only the wt-p53 patients harboring the unmutated IgVH gene were used as a control group in all subsequent survival evaluations. Survival assessed from the time of abnormality detection (or investigation showing wt-p53) was significantly reduced in patients with both missense (P<0.001) and non-missense p53 mutations (P=0.004) in comparison with wt-p53 patients. In addition, patients harboring missense mutation located in p53 DNA-binding motifs (DBM), structurally well-defined parts of the DNA-binding domain, manifested a clearly shorter median survival (12 months) compared to patients having missense mutations outside DBM (41 months; P=0.002) or non-missense alterations (36 months; P=0.005). The difference in survival was very similar in the analysis limited to patients harboring mutation accompanied by del(17p) (n=50). A subset of p53 mutations was identified already at diagnosis. The p53 DBM mutations (n=12) once again resulted in a very short survival of only 17 months (P<0.001; hazard ratio to p53-wt patients 20.8; 95% CI 8.82-48.82); patients with the remaining p53 mutations (n=16) manifested 51 month median survival (P<0.001; hazard ratio to p53-wt patients 5.5; 95% CI 2.41-11.69); a median survival of wt-p53 patients was 110 months. The patients with p53 DBM mutation at diagnosis also manifested very short median time to first therapy (TTFT) (1 month); the remaining p53-affected patients had TTFT 6 months and p53-wt patients 19 months. Summary/Conclusions. The substantially worse survival and the short TTFT suggest a strong mutated-p53 gain-of-function (GOF) phenotype in CLL patients with DBM mutations. The impact of p53 DBM mutations on prognosis and response to therapy should be analyzed in investigatory clinical trials. CLL patients with p53 DBM mutations should be primary candidates for allogeneic stem cell transplantation as their long-term survival is improbable. Supported by grants NS9839-4/2009, NS10439-3/2009, and NS10448-3/2009 (IGA MH, CZ), Research Proposal MSM0021622430 (MEYS, CZ), and the European Research Initiative on CLL (ERIC).
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13q14 DELETION LOAD AND SIZE BOTH CONTRIBUTE TO REFINING PROGNOSIS IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)
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Background. Deletion at 13q14 (del13q) is detected by fluorescence in-situ hybridization (FISH) in about 50% of CLL. Although CLL patients with del13q as the sole cytogenetic abnormality (del13q-only) usually have a good prognosis, more aggressive cases have been reported for del13q-only CLL bearing high percentages of deleted nuclei. Moreover, del13q of different sizes have been described, the prognostic significance of which is still unknown. Aims. to investigate the prognostic significance of del13q by a FISH approach in 342 del13q-only cases and in 265 consecutive unselected CLL cases. Methods. FISH analysis was performed using the following probes: LSI-D13S319 (detecting the D13S319 locus in at least 5% of nuclei (delRB1)), the MIR15A/MIR16-1 region, LSI-BCL11A, LSI-ATM, LSI-p53, CEP12. Results. Maximaly selected log-rank statistics identified the 70% of deleted nuclei as the most appropriate cut-off capable of separating del13q-only cases into two subgroups with different time-to-first-treatment (TTT, p=0.0022). Del13q-only CLL with ≥70% of deleted nuclei showed a shorter TTT than <70% del13q-only cases (median TTT 77 versus 120 months, p=0.0001). One hundred and thirty-five of 342 del13q-only cases (39.5%) had 13q deletions that included the RB1 locus in at least 5% of nuclei (delRB1). Genomic profiles using Affymetrix GeneChip Human SNP6 arrays in 67 cases showed that larger deletions involving the RB1 locus occurred in a proportion of del13q-only cases (34.3%) and were always monoblastic (median size 2,380 Kbp versus 1,200 Kbp for del13q without delRB1). We classified del13q-only cases combining both deletion load and size: i) del13q-only cases bearing del13q in <70% of nuclei without delRB1 (del13q<70%, n=144); ii) del13q-only cases bearing del13q in >70% of nuclei with delRB1 (del13q>70%+delRB1, n=95); iii) del13q-only cases bearing del13q in ≥70% of nuclei without delRB1 (del13q≥70%, n=64); iv) del13q-only cases bearing del13q in ≥70% of nuclei with delRB1 (del13q≥70%+delRB1, n=39). The median TTT of del13q<70% cases (not reached) was significantly longer than the median TTT of del13q≥70%+delRB1 (92 months, p=0.012), del13q≥70% (68 months, p<0.0001) and del13q≥70%+delRB1 cases (82 months, p=0.0025). The presence of delRB1 in del13q<70% CLL was associated with a hazard ratio (HR) for progressive disease of 1.91 (95% CI 1.18-3.08, p=0.008), whereas no additional prognostic information was provided in del13q≥70% cases (HR=0.57, 95% CI 0.50-1.51, p=0.65). In multivariate analysis, the presence of delRB1 increased the risk of progressive disease of del13q≥70% cases (HR=1.69, p=0.036), independently of Rai staging and IGHV status. In 265 consecutive unselected CLL, the presence of del13q in ≥70% of nuclei in the absence of delRB1 identified patients with particularly stable and benign clinical courses (n=48; median TTT not reached). Conversely, del13q≥70%+delRB1 cases (n=24), or patients characterized by a del13q in ≥70% of cells (with or without delRB1, n=25) or a normal karyotype (n=75) had shorter median TTT intervals (range 105-129 months, p<0.01 in all comparisons). Finally, patients bearing trisomy 12 (p=0.48), 11q deletion or 17p deletion (p=0.45) experienced the worst clinical course (p<0.0001). Conclusions. FISH analysis of both deletion load and size allowed to reveal the prognostic heterogeneity of del13q-only cases. A novel prognostic flow-chart involving sequential hybridization with the LSI-D13S319 and LSI-RB1 probes can be proposed.

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FUNCTIONAL CD49D/CD38 MOLECULAR ASSOCIATION IS DETRIMENTAL FOR CLINICAL OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA
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Background. CD49D and CD38 are independent negative prognosticators, whose expressions are often correlated on chronic lymphocytic leukemia (CLL) cells. We recently reported a functional link between CD49D and CD38 as component of a pro-survival circuitry operating in CD49D+/CD38+. This network includes in sequence the CD38/CD26 pair, the CCL3/CCL4 chemokines with the respective receptors, and the CD49D/VCAM-1 axis. Aims. The nature of this circuit was studied by investigating the physical association between CD49D/CD38 and their functions on CLL cell membranes; the validity of the hypothesized prognostic relevance of CD49D/CD38 co-expression was tested in a wide cohort of CLL patients. Methods. The organization of CD49D and CD38 on the membrane of primary CLL cells was investigated by confocal microscopy and biochemical assays. Mec-1, a CD49D+/CD38- cell line derived from CLL cells was used in a comparative way with a clone where CD38 expression was induced by transfection (Mec-1/CD38+). These cells were analysed in terms of adhesion potential. Data on CD49D and CD38 expression along with time-to-first-treatment (TTT) were available for 264 patients. Results. Co-capping and immunoprecipitation experiments in CD49D+/CD38+ CLL cells demonstrated a clear membrane relationship between CD49D and CD38. Co-localization between the molecules was maintained when CLL cells adhered and spread onto VCAM-1 and fibronectin, the CD49D ligands. This finding is an indication that the CD49D/CD38 association is also functional. This issue was answered by designing adhesion assays on VCAM-1-coated plates using the Mec-1 model. Mec-1/CD38+ showed a marked increase in VCAM-1 adhesion compared to Mec-1 (adherent cells relative to control=5.4 vs. 2.3, and 5.4 vs. 1.9 at 15 and 30 min, respectively). Phase-contrast microscopy highlighted the existence of significant differences in the morphology of adherent cells; indeed, Mec-1/CD38+ cells were characterized by a more complex uropod pattern than Mec-1, suggestive of cytoskeleton re-organization. The last tested issue dealt with the influence of CD49D and CD38 co-expression on the network of apoptosis. To this aim, we checked whether adhesion to VCAM-1 might influence the apoptosis promoted by serum deprivation in Mec-1 and Mec-1/CD38+ cells. After 4-5 days of culture on VCAM-1-coated wells without serum, the mean values of Mec-1/CD38+ viable cells (62%±4.8, day 4, and 55%±2.2, day 5) was significantly higher than those observed in the Mec-1 sample (38%±4.2, day 4, and 36%±2.2, day 5; p<0.05). Phase-contrast microscopy highlighted the existence of significant differences in the morphology of adherent cells; indeed, Mec-1/CD38+ cells were characterized by a more complex uropod pattern than Mec-1, suggestive of cytoskeleton re-organization. The last tested issue dealt with the influence of CD49D and CD38 co-expression on the network of apoptosis. To this aim, we checked whether adhesion to VCAM-1 might influence the apoptosis promoted by serum deprivation in Mec-1 and Mec-1/CD38+ cells. After 4-5 days of culture on VCAM-1-coated wells without serum, the mean values of Mec-1/CD38+ viable cells (62%±4.8, day 4, and 55%±2.2, day 5) was significantly higher than those observed in the Mec-1 sample (38%±4.2, day 4, and 36%±2.2, day 5; p=0.001 and p=0.008 respectively). To evaluate the clinical relevance of these observations, we compared the TTT in 564 CLL patients (303 CD49D+/CD38-, 125 CD49D+/CD38+, 95 CD49D-/+CD38, and 41 CD49D+/CD38+). Patients CD49D+/CD38+ were characterized by TTT significantly shorter as compared with patients expressing only CD38 (p=0.01) or CD49D (p=0.01), the longest TTT being however observed in CD49D+/CD38 double negative cases. Conclusions. The association between CD49D and CD38 on the CLL membrane is not only physical, but also functional. The validity of this conclusion is corroborated at a clinical level: the analysis of a simultaneous CD49D/CD38 expression produces a significant refinement of the prognostic potential provided by any of the two factors independently analysed.
INTERACTION BETWEEN ENDOTHELIAL AND CHRONIC LYMPHOCYTIC LEUKEMIA B-CELLS RESCUES FROM APOPTOSIS AND MODULATES GENE EXPRESSION PROFILE OF LEUKEMIC CELLS

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Background. Despite an apparent long life in vitro, CLL cells die rapidly in vitro. This observation suggests that the apoptotic resistance is not intrinsic to leukemia B cells but extrinsic factors are necessary for CLL prolonged survival. Aims. We investigated the interactions between endothelial cells and CLL cells, highlighting molecular networks involved in this cellular crosstalk. Methods. We co-cultured CLL cells on HUVEC endothelial monolayer (HC) or in medium alone (CLL only). Then, we detected CLL viability by flow cytometry and we performed whole-genome high density microarrays. Results. We found that endothelial cells protected CLL from spontaneous apoptosis. After 48h, increased number of alive CLL cells was present in HC condition (59.7 ± 4.2%) compared to CLL alone (22.9 ± 5.1%) (p<0.0001). Moreover, we found that spontaneous in vitro apoptosis was higher in unmutated IGHV CLL (UM-CLL) compared to mutated ones (M-CLL). In HC condition, similar survival was detected between M-CLL and UM-CLL, implying a 2.2-fold increase in relative viability in M-CLL and a 6.1-fold increase in UM-CLL. Moreover, the endothelial cell layer decreased the in vitro sensitivity of CLL cells to Fludarabine-induced apoptotic cell death. The mean viability of CLL cells treated with 10 µM Fludarabine was 19.8% (±4.4%) after 48 hours and 3.8% (±1.3%) after 72 hours. In HC with Fludarabine addition, the mean viability of CLL cells was 37.8% (±9.1%) after 48 hours and 14.3% (±3.2%) after 72 hours. Then, we compared gene expression profiles (GEP) between CLL cultured in contact with EC layer and CLL at baseline to unravel the transcriptional modifications induced by EC cells. Overall 1944 genes were found to be modulated (FC=2, p<0.05). CLL cells in HC condition showed a 22.6-fold increase of CCL2, able to recruit tumor-activated monocytes (FC=4.3, p=0.019) and THBS1 (FC=45.1, p=0.0004) as well as the metalloproteases MMP2 (FC=8.3, p=0.0015) and MMP4 (FC=3.0, p=0.039). The GEP data were confirmed by evaluating the secreted levels of soluble factors in conditioned medium collected after 48h-HC culture. In addition, CLL cells on endothelial layer maintained or increased the expression levels of anti-apoptotic molecules such as stromal cells and macrophages and increase the expression of anti-apoptotic molecules.

ANALYSIS OF AUTOPHagy IN B-CLL SAMPLEs REVEALS INCREASED BASAL AUTOPhagy WHEN COMPARED TO NORMAL B-CELLs

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Background. B-cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in the Western World. It is characterized by the accumulation of long-lived mature B cells. Autophagy is the process of cell component removal whereby cellular elements are encompassed in vesicles and transported to lysosomes for degradation. Autophagy has a role in both cell survival and apoptosis, and is involved in antigen presentation in mature B-cells. Deregulation of autophagy has been implicated in various cancers including leukemia, although it has not been described yet in B-CLL. Aims. To determine if B-CLL patients have recurrent abnormalities in autophagic genes and if so, identify the affect of these abnormalities on the autophagy process. Methods and Results. We tested DNA samples from 212 patients with B-CLL using high-resolution SNP 1M-Duo arrays and analyzed the data using Nexus Biodiscovery software. The results were compared against the Database of Genomic Variants and data from the Wellcome Trust Case Control Consortium, as well as from germ-line samples in selected cases. Amongst the abnormalities noted were recurrent abnormalities involving important autophagy genes (ATG5, ATG6 and ATG7). Whole genome sequencing of two patients with B-CLL also detected mutations in a gene involved in the regulation of autophagy (NOD1). Next we investigated the autophagin flux in B-CLL using the Amnis Imagestream, which combines flow cytometry with cell imaging, allowing simultaneous assessment of morphological characteristics alongside fluorescence signals of large numbers of cells. This makes it an ideal instrument to calculate co-localisation of autophagosomal markers while identifying a subpopulation of cells by their surface markers. We analysed CD5+CD19+ B-CLL cells from eight patients (including one with an ATG5 deletion, 2 with TP53 abnormalities, one with a NOD1 mutation and 4 without genetic autophagic abnormalities) and CD19+ B-cells taken from age-matched controls. Cells were incubated under standard conditions for 2 hours either with or without lysosomal inhibitors prior to staining with antibodies for CD19, CD5, lysosome and LC3. We used the co-localization of LC3 and lysosome as a marker of autolysosomal formation and thereby autophagic flux. Autophagic flux was significantly higher in B-CLL samples when compared with normal B-cells, implying that B-CLL cells have a higher basal autophagy level than normal B-cells. Surprisingly, these increased levels were observed in the B-CLL samples both with and without abnormal autophagy genes. Conclusion. This is the first time that increased autophagy levels have been demonstrated in B-CLL. Investigations to determine whether this pathway might provide a mechanism of survival and perturbation of programmed cell death and therefore represent a new drug target in B-CLL are ongoing.
**Myelodysplastic syndromes - Biology & clinical**

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**GENOMIC ALTERATIONS ASSOCIATED WITH BONE MARROW FAILURE AND PROGRESSION TO LEUKEMIA IN THE BONE MARROW OF DNA-REPAIR DEFICIENT MICE**

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**Background.** Bone marrow (BM) failure may arise from (inherited) mutations or develop through sustained exposure to cytotoxic stresses. Secondary leukemia’s arising from these BM failures are often refractory to current treatments. We focus on genotoxic-stress induced bone marrow failure, such as seen in Fanconi Anemia, Nijmegen breakage syndrome and therapy-related myelodysplastic syndrome/AML. These diseases have a high risk of progression to leukemia. We use a mouse model deficient for Ercc1, an enzyme involved in the repair of interstrand crosslinks (ICLs). In the absence of efficient repair, ICLs result in stalled replication forks and cause cell cycle arrest, mediated by activation of the cell-cycle gatekeepers p16/p19 and/or p53. We have previously shown that Ercc1-deficient mice have a strong reduction in hematopoietic stem- and early progenitor cells (LSK) throughout their lifespan, of ~20 weeks. Our most recent analysis shows that LSKs are reduced in mice deficient for both Ercc1 and p53. Ercc1-deficient mice do not develop leukemia during their lifespan, but when we transplanted Ercc1-deficient BM cells that were heterozygous for Trp53 into lethally irradiated recipients more that 57% of the animals got leukemia and an additional 37% died prior to diagnosis. Thus, our aim is to dissect the early and sequential molecular defects that occur in stem cells from BM failure to pre-leukemia and contribute to the development of overt leukemia.

**Methods.** To monitor genetic changes in different maturation stages we determined gene expression changes in immature (LSK) and mature (BM) hematopoietic cells of 20-week old Ercc1-deficient and control mice. To determine genomic alterations associated with BM failure, we sequenced the exome of the BM 20-week old Ercc1-deficient animals and of Ercc1-deficient fetal liver cells, which contain normal LSK levels. **Results.** Ercc1-deficient BM cells show an up-regulation of the cell-cycle gatekeepers p16, p19 and the p53-targets PUMA and NOXA, which induce apoptosis. In contrast, none of these transcripts were up-regulated in purified LSK cells of Ercc1-deficient mice. Instead, LSK cells showed up-regulation of p21, which is involved in cell cycle arrest and p32-aminogen activator inhibitor -1 (PAI-1), which is a marker for senescence. In the sequences of two exomes of Ercc1-deficient BM we found 1170 and 750 exonic mutations, of which 560 were found in common. These mutations were acquired during the bone marrow failure, as they were not found at the fetal liver stage nor were they present in the mouse SNP database. We will next sequence the leukemia’s arising from Ercc1-deficient BM that is heterozygous for Trp53 to identify leukemia-associated genomic aberrations and compare them to genetic aberrations already present at the BM failure state. 

**Summary/Conclusions.** The gene expression response to ICL-damage in LSK cells is directed at survival by inducing cell cycle arrest/senescence without apoptosis, while in mature cells apoptotic genes are up-regulated. While BM failure is thought to prevent survival of cells with altered genomes, strikingly we found 560 common exonic mutations in two samples of Ercc1-deficient BM. Our mutational analysis is expected to elucidate critical players involved in early and late stages of leukemogenesis from DNA-damage induced BM failure.

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**ACE-536, A MODIFIED TYPE IIIB ACTIVIN RECEPTOR PROMOTES ERYTHROID DIFFERENTIATION IN AN EPO INDEPENDENT MANNER AND PREVENTS ANEMIA IN MYELODYSPLASTIC SYNDROME**

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Myelodysplastic syndromes (MDS) are stem cell disorders characterized by impaired hematopoiesis and cytopenias. The majority of MDS patients present with anemia that is often refractory to treatment with erythropoietin (EPO). As an alternative approach to treating anemia in MDS patients, we have targeted the TGF-β superfamily. This group of molecules has been implicated in erythropoiesis. We have developed a soluble receptor fusion protein consisting of a modified form of activin receptor IIb extracellular domain linked to a human Fc region (ACE-536) that generates a robust increase in RBC’s in several animal models. These preclinical studies is to test whether these models are affected by ACE-536 and to evaluate the efficacy of RAP-536 (murine analogue of ACE-536) in treating anemia in a murine model of MDS. Subcutaneous administration of ACE-536 (10 mg/kg) to C57BL/6 mice resulted in a significant increase in hematocrit, hemoglobin and red blood cells as compared with the vehicle (VEH) group within 4 days. These effects were observed in the presence of an EPO neutralizing antibody suggesting that EPO is not directing the initial RBC response. To investigate the therapeutic potential of RAP-536 in a model of MDS, four month old NUP98-HOX13 transgenic mice (10/dose group) were treated with vehicle (VEH) or RAP-536 (10 mg/kg) twice per week. Wild-type littermates (10/dose group) were dosed with VEH or RAP-536 (10 mg/kg) and used as controls. Prior to the first dose (Day 0), the MDS mice had significantly decreased levels of RBC (6.8%, P<0.05), hematocrit (-8.4%, P<0.05) compared to their wild-type control littermates. After 7 months of dosing, MDS mice treated with RAP-536 had increased RBC counts (+13.8%, P=0.09), hemoglobin (+19.8%, P<0.05) and hematocrit (+14.8%, P<0.05) in comparison to VEH-treated controls. These results demonstrate that ACE-536 may represent a novel therapy for severe anemia for patients with Myelodysplastic Syndrome.

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**MORPHOLOGICAL DIFFERENTIATION OF HYPOCELLULAR REFRACTORY CYTOPENIA OF CHILDHOOD AND SEVERE APLASTIC ANEMIA AND CLINICAL OUTCOME**

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Bone marrow failure syndromes of childhood comprise a heterogeneous group of inherited and acquired hypocellular bone marrow conditions. Severe aplastic anaemia (SAA) and hypoplastic refractory cytopenia (RC), a subtype of childhood myelodysplastic syndrome (MDS) that are the main and most difficult differential diagnoses. In the German childhood SAA 94 study the probability to develop clonal disease, particularly MDS and AML, was 28% after immunosuppressive therapy. To investigate whether the first morphological diagnosis has an impact on the outcome with respect to clonal disease, we established distinct morphological criteria to differentiate between SAA and RC. Only bone marrow trephines and smears with hematopoietic aplasia were diagnosed as SAA, whereas cases even with only small foci of MDS typical morphology i.e. patchy erythropoiesis with defective maturation, in an otherwise adipocytic bone marrow where classified as RC. The participating centers of the European Working Group on Childhood myelodysplastic syndrome (EWO-G-MDS) and German SAA 98 study established a central morphological review. Since introduction of the central review in 1998 the probability to develop clonal disease has continuously dropped to 3% (p<0.01). We performed a
double-blinded inter observer study of 100 different cases of SAA and RCC among 7 hematopathologists. Only in 4 out of 100 cases no agreement could be achieved whether to classify SAA or RCC. The kappa-index was 0.79 indicating that the vast majority of SAA and RCC cases can be reliably differentiated by morphological means only. Our results suggest that the main reason for development of MDS or AML after immunosuppressive therapy of SAA might be the initial morphological differentiation of SAA and RCC. Finally, we found that a clear morphological differentiation reduces the secondary cases of MDS and AML after immunosuppressive therapy significantly.

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A PHASE I STUDY OF PANOBINOSTAT IN COMBINATION WITH 5-AZACITIDINE IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES, CHRONIC MYELOMONOCYTIC LEUKEMIA, OR ACUTE MYELOID LEUKEMIA

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Background. Panobinostat, a pan-deacetylase inhibitor (pan-DACi), has demonstrated anti-tumor activity in patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). Preclinical studies suggest the combination of a hypomethylating agent such as 5-azacitidine (5-aza) and a pan-DACi may, at least in part through gene studies suggest the combination of a hypomethylating agent such as 5-azacitidine (5-aza) and a pan-DACi may, at least in part through gene

Results. 18 patients, median age 69 years (range 34-80), have been enrolled to date including 13 with MDS, 3 with AML, and 2 with CMML. Patients were evaluated at 3 different panobinostat doses: 6 (20 mg), 5 (30 mg), and 7 (40 mg). The AEs analysis is based on currently available data of all 18 patients. AEs of all grades, regardless of study drug relationship, included nausea (12 [67%]), vomiting and diarrhea (11 [61%]), fatigue (10 [56%]), decreased appetite (9 [50%]), and asthenia (9 [38%]). Grade 3/4 treatment-related AEs included thrombocytopenia (4 [22%]) and febrile neutropenia (3 [17%]). Serious AEs, regardless of study drug relationship, included febrile neutropenia and asthenia (4 each [22%]). One dose-limiting toxicity (DLT) was observed (grade 4 febrile neutropenia) in the 20 mg panobinostat cohort and two DLTs (grade 3 hyperbilirubinemia; grade 3 nausea and asthenia) in the 40 mg cohort. Summary/Conclusions. Current data show that the addition of panobinostat to 5-aza therapy is safe with no unexpected toxicities. To date, the most common grade 3/4 AEs are gastrointestinal events and fatigue. The most common grade 3/4 treatment-related AEs include febrile neutropenia and thrombocytopenia, with one DLT observed in the 20 mg cohort and two in the 40 mg cohort. Based on the occurrence of two DLTs and one withdrawal of consent following grade 3 fatigue in the 40 mg cohort, the decision was made to enroll additional patients at the lower dose level to further assess safety and tolerability at 30 mg. In parallel, the ongoing patients at 40 mg are monitored for long term tolerability. Updated data, including preliminary efficacy data, will be presented at the meeting. Backgrounds. Myelodysplastic syndromes (MDS) are affecting mainly elderly patients, and age is considered per se a negative prognostic factor. Most patients have comorbidities that presumably impact negatively overall survival and quality of life, and may influence response to therapy. It was recently demonstrated that MDS patients aged > 75 yrs treated with azacitidine have a significantly longer overall survival respect to best supportive care treated patients. Aims. We wanted to analyze whether the presence of comorbidities could have an impact on survival, response and management of azacitidine treatment in MDS patients, in clinical practice. We also evaluated patient outcome, type of response according to IWG criteria 2006, as well as adverse events and cause of death. Methods. We analyzed 103 MDS patients (IPSS: 30% INT-1, 49% INT-2 and 21% High) treated with Azacitidine 75 mg/m²/day sc for 7 days every 28. Mean number of cycles was 9 (range 1-42). Mean age was 69 yrs (50-82), 50% of patients were >= 75 yrs and 59% of the latter >= 80 yrs; 71% of patients were male. Patients were evaluated by three different geriatric score: Charlson comorbidity index (CCI) (54% of patients scored 0, 37% of patients 1 or 2, and 9% of patients >= 3), the Cumulative Illness Rating Scale (CIRS) (37% of patients scored 0, 57% of patients 1 and 26% of patients >= 2) and the Adult Comorbidity Evaluation-27 (ACE-27) (41% of patients scored none, 29% of patients mild, 23 % of patients moderate and 7% severe). The OS of patients treated with azacitidine was compared with that of a diagnosis- and age- matched untreated control group of patients (n= 246) (Italian registry of MDS - AISSM) in whom comorbidities had been evaluated by CIRS score. Results. Median overall survival (OS) of our patient cohort was 22 months. Median OS in patients < 75yrs (25 months) and >= 75 yrs (15 months) was not significantly different (p value > 0.160). No correlation was present between comorbidity scores, age and hematological response. OS was strictly depending on scores. OS in patients with higher CCI, CIRS, and the ACE-27 was respectively 6.5 months, 10 months and 8 months vs OS in patients with lower CCI, CIRS and ACE-27 was respectively 20, 22 and 22 months. Overall response rate (HR, CR and PR) was 49%, stable disease was obtained in 57 of MDS patients. IWG responses did not correlate with age, sex and comorbidity scores. Hematological or non-hematological adverse events grade III and IV were presented by 34% and 36% of patients, respectively. Adverse events were uniformly distributed independently from age. Conclusions. MDS patients with comorbidities may be treated with success with azacitidine, without any substantial increase in AE and with improvement of OS respect to untreated patients. Nevertheless, comorbidities per se negatively influence OS. Evaluation of comorbidities with validated indexes is an useful and easily applicable tool to refine prognostic evaluation and should be include routinely in patient assessment.
DNMT3A MUTATIONS IN MYELOPROLIFERATIVE NEOPLASMS

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Background. An increasing number of gene mutations is being identified in the ‘classic’ myeloproliferative neoplasms (MPN) essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). For instance, use of array-based techniques led to the identification of mutations in candidate genes involved in epigenetic regulation such as TET2, ASXL1, and EZH2. Most recently, a whole-genome sequencing study in acute myeloid leukemia (AML) uncovered recurrent mutations in 22% of AML patients in another epigenetic regulator, the DNA methyltransferase 3A gene DNMT3A (Ley et al., N Engl J Med 2010, 363: 2424-2435). Aim. To explore mutations of DNMT3A in a series of 115 well-characterized chronic- and blast-phase MPN cases: ET, n=36; PV, n=36; PMF, n=18; secondary MF (SMF), n=4; AML secondary to MPN (aAML), n=35. Methods. All coding exons of DNMT3A (2-25) were analyzed using direct DNA sequencing. Mutation data on JAK2 V617F, MPL, W515L, CBL (exon 8-9), TET2 (exon 5-11), ASXL1 (exon 12), EZH2 (exon 2-20), and IDH1/2 (exons 4) as well as SNP array data were available in all cases. Results. In a total of 12 heterozygous DNMT3A sequence variants were identified resulting in an overall frequency of 10% (12/115). DNMT3A alterations were most frequently detected in SMF (50%, 2/4) and aAML (17%, 6/35), followed by PV (7%, 2/30), PMF (6%, 1/16), and ET (5%, 1/30); they consisted of 8 nucleotide substitutions and 4 frameshift deletions (P264sf, W305sf, R488sf, and D766sf). 2 nucleotide substitutions resulted in direct stop codons (E523* and E477*), whereas 6 represented missense alterations; of these, the amino acid residue R882 was recurrently affected in 4 cases (R882H, n=3; R882C n=1). Somatic origin was confirmed in 5 cases including 2 known (E477* and R882H) and 3 novel missense alterations; of these, the amino acid residue R882 was recurrently affected in 4 cases (R882H, n=3; R882C n=1). Somatic origin was confirmed in 5 cases including 2 known (E477* and R882H) and 3 novel missense alterations; of these, the amino acid residue R882 was recurrently affected in 4 cases (R882H, n=3; R882C n=1). Furthermore, none of the cases harbored MPL, CBL, TET2 or EZH2 mutations. In contrast, DNMT3A alterations occurred concurrently with JAK2 (40%, 7/12), IDH1/2 (33%, 4/12), and ASXL1 (8%, 1/12) gene mutations. In SNP array analysis, no distinct patterns of concurrent genomic aberrations could be identified. In terms of clinical data, none of the ET/PV cases showed a correlation between the identified mutations and clinical phenotype. Conclusions. Our data underscore the increasing complexity of MPN pathogenesis due to additional mutations in candidate genes involved in epigenetic regulation such as DNMT3A and TET2. DNMT3A and TET2 gene mutations in the context of MPN cases should be considered for clinical studies investigating potential targeted therapies.
Table 1.

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<th>Patients</th>
<th>JAK2V617F</th>
<th>JAK2V617F After IFNa</th>
<th>JAK2V617F % evolution</th>
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G6 ALLEVIATES JAK2-V617F MEDIATED MYELOPROLIFERATIVE NEOPLASIA BY PROVIDING SIGNIFICANT THERAPEUTIC EFFICACY TO THE BONE MARROW

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The discovery of the Jak2-V617F mutation in a large percentage of myeloproliferative neoplasm (MPN) patients has been a driving force behind the development of small molecule Jak2 inhibitors. Unfortunately, most drugs have been found to be merely palliative as they have little to no efficacy in the bone marrow and, therefore, cannot alter the natural history of the disease. We recently developed a small molecule Jak2 inhibitor called G6 and found that in a xenograft model of Jak2-V617F mediated MPN. We implemented such a study and found that G6 provided therapeutic benefit to the peripheral blood in this model as determined by elimination of leukocytosis, thrombocytosis, and erythrocytosis. With respect to the spleen, G6 provided marked therapeutic efficacy as measured by normalization of spleen size and elimination of megakaryocytic hyperplasia. In the critically important bone marrow, G6 normalized the pathologically high levels of pJAK2 and pSTAT5. It significantly reduced the megakaryocytic hyperplasia in the marrow and completely normalized the M:E ratio. Most importantly, G6 selectively reduced the mutant Jak2 burden by 67% on average with virtual elimination in one-third of all treated mice. This significant reduction in the Jak2 mutant burden correlated with the presence of G6 in the plasma. Lastly, clonogenic assays using marrow stem cells from the MPN mice revealed a time-dependent elimination of the clonogenic growth potential of these cells by G6. Collectively, these data indicate that G6 exhibits exceptional efficacy in the peripheral blood, spleen, and most importantly, in the bone marrow. As such, G6 appears to alter the natural history of Jak2-V617F mediated MPN.

References

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NF-E2 MEDIATES EXPRESSION OF THE CYTOKINE IL-8, AN INDEPENDENT PREDICTOR OF INFERIOR OUTCOME AND PRESENCE OF CIRCULATING BLASTS IN PMF PATIENTS

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Background. The transcription factor nuclear factor erythroid-2 (NF-E2) is overexpressed in patients with myeloproliferative neoplasms (MPN) irrespective of the presence of the JAK2V617F mutation.12 We have recently engineered a transgenic mouse model which recapitulates many features of MPN including thrombocytosis, leukocytosis, typical bone marrow features and transformation to acute leukemia14 demonstrating a role for NF-E2 overexpression in the pathophysiology of MPN. Because the targets mediating NF-E2 effects are not well characterized, we conducted microarray analysis of CD34+ cells transiently transduced to overexpress NF-E2 or to silence NF-E2 via shRNA, in order to identify novel NF-E2 target genes. Aims. To identify novel target genes of the transcription factor NF-E2. Methods. Peripheral blood CD34+ cells from healthy donors were lentivirally transduced with a NF-E2 cDNA or with a shRNA directed against NF-E2. Cells were FACS sorted to obtain pure populations of transduced cells and assayed for gene expression using an Affymetrix Exon-Array ST 1.0. IL-8 mRNA and protein expression were investigated by qRT-PCR of independently transduced CD34+ cells, by intracellular FACS staining and by ELISA. The proximal 262 bp of the IL-8 promoter were used in a luciferase reporter gene assay. Results. Transduction of CD34+ cells with the NF-E2 cDNA induced IL-8 mRNA expression 3-4 fold, while transduction of an shRNA against NF-E2 lowered IL-8 mRNA expression by 50% (p<0.008 by repeated measures ANOVA). Concurrently, IL-8 protein expression in the supernatant increased and decreased 1.7- and 2-fold, respectively. This effect was reproducible in the U937 cell line, where NF-E2 transduction also significantly increased IL-8 production, as measured both by ELISA and intracellular FACS staining (p<0.005 and p<0.02, respectively). Co-transfection of the proximal 262 bp of the IL-8 promoter with both NF-E2 and its obligate heterodimeric partner MaF-G, but not with either subunit alone, increased reporter gene activity 3-fold (n=3; p<0.001). NF-E2 binding to the IL-8 promoter in vivo was previously demonstrated by ChIP assay in K562 erythroleukemia cells. Summary/Conclusions. We have identified IL-8 as a novel NF-E2 target gene. Serum levels of IL-8 are elevated in both PV and PMF patients.5,6 Recently, increased IL-8 levels have been shown to be predictive of inferior survival in PMF patients in multivariate analysis.3,4 Likewise, elevated IL-8 levels were associated with the presence of >1% circulating blasts.2 We therefore propose that one mechanism through which aberrant NF-E2 expression in MPN patients exerts its pathophysiologic effect is by increasing IL-8 production.
Acute leukemia - Cytogenetics & genomics

1065 INTEGRATED TRANSCRIPT AND GENOME ANALYSES REVEAL NKX2.1 AND MEF2C AS NOVEL ONCOGENES IN T-CELL ACUTE LYMPHBLASTIC LEUKAEMIA


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Background. T-lineage acute lymphoblastic leukemia (T-ALL) is a malignancy of thymocytes. T-ALL represents about 15% of pediatric ALL cases but has an inferior outcome compared to B-ALL as approximately 30% of T-ALL cases relapse during therapy or within the first 2 years following treatment and eventually die. T-ALL is mostly characterized by genetic abnormalities that are crucial for T-cell pathogenesis. Various genetic rearrangements in T-ALL occur in a mutually exclusive pattern (such as TLX1, TLX3, TAL1 and LMO2 rearrangements) and these are identified in ~60% of pediatric T-ALL cases. For the remaining 40% the underlying oncogenic rearrangements remain unresolved. Aim: To identify novel oncogenic pathways in T-cell acute lymphoblastic leukemia (T-ALL) cases that lack a currently known (mutually exclusive) oncogenic rearrangement. Methods. We combined expression profiling of 117 pediatric patient samples and detailed molecular cytogenetic analyses including the Chromosome Conformation Capture Capture on Chip (4C), arrayCGH, FISH and LM-PCR. Results. In a supervised cluster analysis based on the gene expression data, two T-ALL subtypes were identified that lacked rearrangements of known oncogenes, both comprising approximately 10% of the 117 pediatric T-ALL cases studied (n=12 in both groups). One subtype associated with cortical arrest, expression of cell cycle genes and ectopic expression of cell cycle genes and ectopic expression of cell cycle genes (n=12 in both groups). One subtype associated with cortical arrest, expression of cell cycle genes and ectopic expression of cell cycle genes. In this subgroup 7 out of 12 cases carried LMO2 and LYL1, HHEX and the MEF2C transcription factor. In this subgroup we found several genetic rearrangements that all converge on the upregulation of MEF2C, a gene involved in muscle development. We also demonstrated by ChIP and knock-in and knock-out models, that MEF2C is a transcription factor that binds the promoters of LMO2 and HHEX and is responsible for the expression of cell cycle genes. Knockdown of MEF2C also induced cellular differentiation in a cell line model. Furthermore, in cellular transformation assays using NIH-ST1 and BJ-EHT cells we could demonstrate transforming potential for MEF2C as well as NKX2-1 in combination with RAS or MYC oncogenes. Summary/Conclusions. We propose NKX2-1, NKX2-2 and MEF2C as novel T-ALL oncogenes that are activated by various rearrangements.
deletions were independent risk factors. The 4-year OS rates for these aberrations were: CRLF2-d 15% (95% CI: 4.8-24.5%); ICHEL-t 29% (16-44%); PAX5 deletions 58% (44-71%). In conclusion, micro-deletions of key B-cell differentiation and cell cycle control genes are highly prevalent in adult ALL but vary in frequency by genetic subgroup. There was evidence to suggest that deletions of B-cell differentiation genes were linked to outcome. CRLF2-d and ICHEL-t represent distinct and prevalent subgroups of adult ALL which are associated with a poor outcome.

1067 GENE EXPRESSION BASED OUTCOME PREDICTION IN CYTOGENETICALLY NORMAL AML: A MULTICENTER APPROACH FOLLOWED BY INDEPENDENT VALIDATION SHOWS PROOFS CLINICAL PROVENTION

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Background. Cytoogenetically normal acute myeloid leukemia (CN-AML) is biologically and clinically heterogeneous. During recent years genomic aberrations and deregulated gene expression signatures have been identified to provide important prognostic information. Aims. Our intention was to assess whether gene expression-based outcome prediction using novel biostatistical approaches applied to large data sets generated across four different laboratories could refine previous prognostic signatures in CN-AML. Methods. We generated gene expression profiles of 220 clinically well annotated CN-AML cases in a multicenter setting, comprising three different expert laboratories. For the analysis Affymetrix Human Genome U133plus2.0 Arrays and a standardized labeling protocol were used. Following data normalization and correction of batch artifacts, we applied L1-penalized Cox proportional hazards regression to develop a sparse prognostic model for overall survival. Our model was then validated by (i) leave-one-out cross-validation and (ii) by evaluating the prognostic accuracy in two independent CN-AML data sets (n=163 and n=79 cases) generated in a fourth laboratory. Results. We identified a 13-gene signature for overall survival that was successfully validated by means of cross-validation (P<0.001, Hazard Ratio (HR) 1.56, 95% Confidence Interval (CI) 1.21-2.00) as well as in both independent data sets (P<0.001, HR 1.85, 95%CI 1.40-2.46; and P=0.004, HR 1.85, 95%CI 1.22-2.81, respectively) using a Cox Proportional Hazards model. The gene signature contained prognostically informative features of both high and low risk status, and it was associated with younger age (P=.042), female gender (P=.019), and higher WBC (P=.019). In addition, it was validated in two independent data sets (n=163 and n=79 cases), where the 13-gene signature retained its prognostic relevance in a multivariable model adjusting for age, FLT3-ITD and NPM1 alterations (P=.007, P=.004 and P=.013). Hence, we were able to prove the clinical value of our gene signature in a multicenter setting.

1068 RECURRENT NADH DEHYDROGENASE SUBUNIT 4 (ND4) MUTATIONS IN ACUTE MYELOID LEUKEMIA

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Background. Recently, mutations in NADH dehydrogenase subunit 4 (ND4), a mitochondrial encoded transmembrane component of the electron transport chain respiratory Complex I, have been described in acute myeloid leukemia (AML). Aims. In the present study, we investigated the prevalence and prognostic importance of ND4 mutations in 452 AML patients. Methods. After obtaining informed consent according to the Declaration of Helsinki, DNA from diagnostic bone marrow or peripheral blood samples were analyzed from 452 adult patients (aged 17-60 years) with de novo (n=402) or secondary AML (n=50). Mutations were identified using a PCR-based mutation screening approach. Results. Homoplastic and heteroplastic ND4 mutations (e.g. mutations affecting all or only a fraction of mitochondrial DNA copies) predicted to affect translation were detected in 34 of 452 patients (7.5%). Thirty-one cases had single point mutations resulting in amino acid substitutions, two cases had two separate missense ND4 point mutations, and another case had a single base pair deletion predicted to result in a truncated protein. ND4 mutations were associated with younger age (P=.042) and NPM1 mutations (P=.019), but were inversely associated with NPM1 mutations (P=.039). Analysis of buccal swab samples available for three ND4mutated patients demonstrated that two heteroplastic mutations were somatic and one homoplastic mutation was germline. In six additional patients (five with heteroplastic and one with homoplastic ND4 mutations), we sorted T-cells (CD3+CD45brightCD34-) and leukemic blasts (CD3+CD45~) from diagnostic leukemia samples. In the five heteroplastic ND4mutated cases, we detected only wildtype ND4 in the healthy T-cell population, while the ND4 mutational load was restricted to the leukemia blasts in all five cases, thus confirming the somatic nature of these mutations. In contrast, the homoplastic ND4 mutation was detected in both the healthy T-cell and the leukemia blasts populations, demonstrating that this is a germline mutation. Additionally, analysis of ND4 mutation status in complete remission samples revealed loss of the ND4 mutation in 4/5 AML cases where patients had heteroplastic ND4 mutations at diagnosis, while the same was true for only 1/7 cases who presented with homoplastic ND4 mutations. Although univariate analysis demonstrated similar relapse-free (RFS; P=.676) and overall survival (OS; P=.948) for ND4mutated patients (n=54) compared to ND4wildtype patients (n=418), comparison by the logrank test according to the heteroplasmy status of ND4 mutations revealed longer RFS (P=.025) and OS (P=.012) for patients with heteroplastic ND4 mutations (n=12). Conclusions. These observations extend our knowledge of ND4 mutations and further establish the link of mitochondrial mutations with leukemia. Our data suggests that heteroplastic and homoplastic ND4 mutations may elicit different effects in leukemia biology, such as contribution to leukemia maintenance or sensitization of leukemia cells to therapy.
Acute lymphoblastic leukemia - Clinical

1070 PROGNOSTIC SIGNIFICANCE OF LEF1 EXPRESSION IN ADULT B-PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. The Wnt signaling pathway is linked to proliferation and survival of leukemia cells and has emerged as a potential target for antileukemic therapy. Lymphoid Enhancer Factor (LEF) 1, a key mediator of the Wnt pathway, was shown to be involved in leukemia transformation. Recently, microdeletions and mutations of the LEF1 gene were identified in pediatric T-cell acute lymphoblastic leukemia (ALL). Aims. To further investigate the yet undefined role of LEF1 in B-lineage ALL, we determined the prognostic impact of LEF1 expression in adult B-precursor ALL patients. Methods. LEF1 mRNA expression was determined by quantitative real time PCR in pretreatment bone marrow samples of 282 adults with newly diagnosed B-precursor ALL enrolled on the 06/99 (n=138) and 07/03 (n=144) GMALL multicenter trials. For statistical analyses, patients were grouped into quartiles according to LEF1 expression levels (LEF1 high (Q4; n=71); LEF1 low (Q1-3; n=211)). The median follow-up for living patients was 42.6 months. Results. Patients who received allogeneic stem cell transplantation in first remission were censored at the time of transplantation for survival analyses. Multivariate analyses were performed according to the Cox proportional hazards model, including the following variables in the full model: white blood cell (WBC) count (10/ul increase), age (10-year increase), CD20 positivity, BCR-ABL1, MLL-AF4, and immunophenotype. We focussed our analysis on standard risk (SR) patients as defined by GMALL (BCR-ABL1- and MLL- AF4-negative patients with CR after first induction therapy and WBC ≥30/ul; n=91), as this patient group lacks molecular markers for further risk stratification. To evaluate the presence of LEF1 mutations, we performed DNA sequencing of LEF1 exons 2 and 3, as the observed hot spot regions in T-ALL, in 41 B-precursor ALL patients. Results. No significant differences regarding age, WBC, the immunophenotype, or GMALL risk groups were found between the two LEF1 groups. High LEF1 expression was associated with CD20 positivity (P=0.01) and inversely associated with the expression of myeloid markers (P=0.001). Patients with high LEF1 expression had a significantly shorter relapse-free survival (RFS), compared to low LEF1 expressers (5-year RFS: LEF1 high: 29%; LEF1 low: 51%; P=0.005). In multivariate analyses, LEF1 was independently predictive for RFS (hazard ratio 1.9 [95% CI 1.1-3.3]; P=0.02), the other factors in the final model were WBC, age, and immunophenotype. Similarly in SR patients, high LEF1 expression was associated with a significantly inferior RFS (5-year RFS: LEF1 high: 39%; LEF1 low: 61%; P=0.01). Upon Cox analysis, LEF1 expression was the only factor with prognostic significance for RFS [hazard ratio 2.8 [95% CI 1.2-6.3]; P=0.01] in SR patients. The mutational analyses revealed no LEF1 mutations in exons 2 and 3, suggesting that in B-precursor ALL transcriptional activation of LEF1 rather than inactivating mutations as in T-ALL might play a role. Conclusions. High LEF1 expression identifies adult B-precursor ALL patients with significantly inferior RFS, supporting a pathogenic role of Wnt signaling in ALL. Thus, determination of LEF1 might improve pretreatment risk assessment in SR ALL patients. Moreover, patients with high LEF1 expression may be considered for new molecular therapies, including agents targeting the Wnt pathway.

1071 DEXAMETHASONE VERSUS PREDNISOLONE FOR THE TREATMENT OF ADULT ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Corticosteroids are essential and one of the mainstays in the treatment of acute lymphoblastic leukemia (ALL). The current randomized comparisons between dexamethasone (DXM) and prednisolone (PND) in childhood ALL indicated a statistically significant

into 7 distinct amplicons (median length: 342 bp). NGS data were analyzed using GS Variant Analyzer Software 2.3 (454 Life Sciences) and Sequence Pilot version 3.4 (JSI Medical Systems, Kippenheim, Germany). In median, 821 reads per amplicon and patient (range 217-1687) were obtained, thus yielding a sufficient coverage for mutation detection with high sensitivity (<5%). Overall, 15 mutations were detected in 13 patients: 7 missense alterations, 1 nonsense mutation, 6 frameshift alterations, and 1 in-frame insertion. RUNX1 mutations were distributed across several exons, but like in myeloid malignancies predominantly clustered in the RUNT domain (aa 50-177; 11/15 mutations) and TAD domain (aa 291-371; 4/15 mutations). In the cohort of B-ALL (n=52 including 22 cases with BCR-ABL1 rearrangement), only 2 cases (3.8%) were RUNX1-mutated. Both of them harbored a BCR-ABL1 rearrangement. In contrast, in T-ALL 13 distinct mutations were found in 13 patients: 7 missense alterations, 1 nonsense mutation, 6

Corticosteroids are essential and one of the mainstays in the treatment of acute lymphoblastic leukemia (ALL). The current randomized comparisons between dexamethasone (DXM) and prednisolone (PND) in childhood ALL indicated a statistically significant

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and clinically important decrease in rate of isolated central nervous system (CNS) relapse and an increase in event-free survival (EFS) with DXM. But the data were limited in adult ALL. Aims. We evaluate the role of DXM compared to PDN during induction or subsequent phases of therapy in adult ALL patients. Methods. From January 2000 to January 2010, ninety five standard risk (SR) and 132 high risk (HR) or very high risk (VHR) newly diagnosed adult ALL patients entered the randomized trial of DXM at 6 mg/m²/d vs PDN at 600 mg/m²/d with ALL treatment protocol. HR ALL was defined as patients fulfilling at least one of the following criteria: age 35 years and older, WBC count greater than 30x10⁹/L for B-lineage ALL or more than 100x10⁹/L for T-lineage ALL at diagnosis, time to complete resolution (CR) of less than 4 weeks. Patients who had none of these risk factors were considered as SR, and patients with cytogenetics of t (9; 22) or BCR-ABL fusion gene positive were considered as VHR. Results. The median follow-up time was 3.5 years. There were no significant differences in terms of CR between the PDN and DXM arms, no matter in SR group (64% vs 57%, p=0.55) or in HR-VHR group (72% vs 7%, p=0.42). In patients with SR, the cumulative incidence of isolated CNS relapse was lower for DXM patients than for PDN patients, with 3-year cumulative estimates of 7.5% and 12.1%, respectively (p=0.015). The 3-year disease-free survival (DFS) and 3-year overall survival (OS) rates were 49% and 52% in SR patients who received DXM which was significantly higher than 40% and 46% in SR patients who received PDN (p=0.005, 0.01). For patients with HR-VHR, the isolated CNS relapse rate was similar for patients assigned to DXM compared with that of patients assigned to PDN (3-year cumulative estimates: 11.6% vs 14.7%, p=0.15), and there were no differences for 3-year DFS rates (32% vs 30% vs 0.59) and 3-year OS rates (58% vs 53%, p=0.36) based on blast type (DXM vs PDN). During induction the incidence of grade 3-4 infection was similar in the two arms (DXM 18.5% vs PDN 15.3%, p=0.20), and during post induction consolidation, it was higher in the DXM arm (15.3% vs 8.9%, p=0.02). Reversible hyperglycaemia and myopathy were higher in the DXM arm than in the PDN arm (5.8% vs 4.8%, 4.2% vs 3.1%), but the differences were not statistically significant (p=0.5, 0.35). Conclusions. Our results indicate that DXM might have benefit in adult SR ALL patients, as it was proved in children, especially concerning the long-term survival and the CNS relapse. But for patients at HR of relapse, it is unlikely that only modification of steroids replacementwould alter their dismal prognosis.

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LONG-TERM FOLLOW UP OF ADULT PATIENTS WITH NEWLY DIAGNOSED PHILADELPHIA-POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (PH+ALL) RECEIVING IMATINIB AND CHEMOTHERAPY AS FRONT-LINE TREATMENT WITHIN GMALL STUDIES

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Background. Imatinib (IM) in combination with chemotherapy regimens followed by allogeneic SCT has become the standard treatment for younger patients with Ph+ ALL. Complete remission (CR) rates generally exceed 90%. The efficacy on the long-term outcome data obtained from a large group of prospectively evaluated patients is still unclear. Aims. The aim of this analysis was to analyze the efficacy of an early imatinib administration started simultaneously with induction chemotherapy according to the GMALL protocol 07/103 (A3). These results were compared with two consecutive patient cohorts of the GMALL study group, where IM was given alternating and/or concurrent, which has been previously reported by Wassmann et al. (Blood 2006;108:1469). Methods. A total of 335 patients with newly diagnosed Ph+ ALL who received IM at a single daily oral dose of 600 mg within 5 successive treatment cohorts were compared. A1: IM was given simultaneously with start of induction (IND) and first consolidation (CONS1) and again after CONS1 (n=51); A2: IM was given during the second half of IND and then continued throughout CONS1 until SCT (n=105); A3: IM has been initiated together with start of induction chemotherapy and continued throughout CONS1 until SCT (n=179). Minimal residual disease (MRD) was serially assessed by quantitative RT-PCR, mutational analyses was performed by D-HPLC and direct sequencing. Results. The median age of all patients was 48 years (17-65), 57(17)% patients were patients 55 years of age or older. CR rates in cohorts A2 and A3 were 89.4% and 85.7%, induction deaths occurred in 5.8% and 11.5% of patients, treatment failure was observed in 4.3% and 3% of pts., respectively. The molecular response rate based on PCR negativity for bcr-abl transcripts after CONS1 was superior in cohort A3 with 33% (26/79) as compared to 12.5% (5/40) and 4.2% (2/47) in cohorts A2 and A1, respectively (p=0.01). Overall treatment outcome improved with earlier initiation and more prolonged administration of IM in the three successive cohorts. Event-free survival (EFS) and overall survival (OS) at 4 years was 31%, 40% and 50% in cohorts A1, A2 and A3, respectively. To date, 219 patients (66.4%) underwent SCT in CR1 (A1: n=39; A2: n=74; A3: n=106), with a median age of 39.5 years. The 3 year probability of DFS of pts. in cohort A3 who received myeloablative conditioning regimens combining TBI with cyclophosphamide or etoposide was 72. The incidence of relapse after SCT was lower among patients in cohort A3 (11.3%) than those in A2 (24.3%) or A1 (30.8%). For all pts. transplanted in CR1 irrespective of treatment cohort, median OS was 57% after 5 yrs. and 52% after 7 yrs. Patients who did not undergo SCT in CR1 had a dismal outcome, with a median OS of 9.4 months and 14% alive after 3 years. Conclusion. In conclusion, intensive chemotherapy and early administration of IM is feasible in patients with newly diagnosed Ph+ALL and is associated with superior treatment outcomes after SCT. SCT in CR1 remains the treatment of choice even in patients who achieve a good molecular response to initial therapy.

1073
PROMISING RESULTS OF SPECIFIC IMMUNOCHEMOTHERAPY IN BURKITT'S LEUKAEMIA OR LYMPHOMA REGARDLESS OF THE HIV INFECTION STATUS: RESULTS OF A PHASE II STUDY
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Background and aims. The addition of rituximab to specific chemotherapy for Burkitt's leukemia or lymphoma (BL) has yielded promising results in phase II studies. However, most of these studies excluded patients infected by the human immunodeficiency virus (HIV). The results and toxicity of a phase II study including patients with BL regardless of their HIV status are presented. Methods. This trial (Burkimab) derived from the B-ALL/NHL2002 (German Multi-center Adult Acute Lymphoblastic Leukaemia Group, GMALL) consisted of a prephase with cyclophosphamide and prednisone, followed by cycles (A, B, C) including rituximab, ifosfamide, high-dose methotrexate and cytarabine. Patients in localized stages (I, II non bulky) received 4 cycles (A1+B1+C1 and A2) and those with bulky stage II or in advanced stages (III, IV) or Burkitt's leukemia received 6 cycles (A1, B1, C1, A2, B2, C2), plus two additional doses of rituximab. Patients 55 yr. received only A and B cycles, with 50% reduction in doses of methotrexate and cytarabine. Informed consent was obtained from all patients (MRI). 121 patients were included (2004-2010). Median age was 45 yr. (range 15-85), 97 (80%) were in advanced stages (III-IV), with extranodal involvement ≥2 sites in 56 (46%) and Burkitt's leukemia in 24 (20%). LDH was elevated in 108 (91%), ECOG score was ≥2 in 54 (45%), and age-adjusted IPI was: 450 | haematologica | 2011; 96(s2)
low 6 (5%), low-intermediate 17 (14%), intermediate-high 48 (41%) and high 47 (40%). The only difference between HIV- (n=80) and HIV+ (n=41) cohorts was age (48 vs. 42 yr, respectively, p=0.03). Seventy-three (HIV-) and 40 (HIV+) patients were evaluable for treatment results. Complete response (CR) was achieved in 63 (86%) vs. 33 (83%), 4 (5%) vs. 5 (15%) died during induction and 4 (5%) vs. 2 (5%) did not respond. The remaining 2 patients (HIV+) were removed early from the trial. After a median follow-up of 2 yr. (range 0.4-7), 4 relapses (5%) vs. 2 (5%) were observed (isolated BM: 2, isolated CNS: 2, combined BM+CNS: 1, extranodal: 1) and 3 (4%) vs. 5 (13%) patients died in remission during chemotherapy (p=0.12). The 4-yr. OS probabilities were 79% (95%CI, 69%-89%) vs. 67% (95%CI, 52%-79%) (p=0.11), the 4-yr. DFS probabilities were 90% (95%CI, 85%-98%) vs. 79% (95%CI, 65%-93%) (p=0.12), and the 4-yr. EFS probabilities were 76% (95CI, 66%-86%) vs. 62% (95%CI, 47%-77%) (p=0.17). For patients aged ≤55yr. grade 3-4 hematological toxicity, mucositis and infections were significantly more frequent in HIV+ patients either as a whole or considering cycles A, B and C separately. For patients aged ≥55yr. only grade 3-4 neutropenia in A cycles was significantly more frequent in HIV+ patients. Conclusions. This phase II trial showed promising results of specific immunotherapy in adult patients with BLL. Although hematological, mucosal and infectious toxicity was higher in HIV+ cohort, the CR, DFS, OS and EFS were not significantly different according to HIV infection status. Supported in part by José Carreras Leukämie-Stiftung e.V. Cooperation project DJCLS H 06/05 (GMALL-PETHEMA), RD06/0020/1056 from RTICC, Instituto de Salud Carlos III, and Joseph Carreras Research Institute, Spain.

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GENOMIC VS. GENE EXPRESSION-BASED METHODS IN THE DETECTION OF IKAROS (IKZF1) ALTERATIONS AND EVALUATION OF THEIR PROGNOSTIC IMPACT IN CHILDHOOD ALL

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Background. Recently, Ikaros (IKZF1) gene deletions have been described as adverse prognostic factors in childhood acute lymphoblastic leukemia (ALL). Nevertheless, there is still lack of data on IKZF1 impact concerning different treatment protocols, minimal residual disease (MRD) and the most suitable diagnostic method in BCR/ABL-negative ALL. Aim. To compare the DNA- vs. RNA-based approach assessing Ikaros status in a cohort of children with BCR/ABL-negative ALL treated by non-MRD based protocol ALL IC-BFM 2002. Methods. A) Gene expression of functional (Ik1, Ik2) vs. short (Ik4, Ik4A, Ik4del, Ik6, Ik6del, Ik8) IKZF1 isoforms was evaluated using Lab-on-a-chip (Agilent) electrophoresis and reported either as an absolute level or relative to the total signal. Thresholds for abnormal expression were set based on the analysis of peripheral blood of healthy donors, remission bone marrow (BM), and sorted B- and T-cell precursor subpopulations. B) MLPA (multiplex ligation-dependent probe amplification) was performed on BM DNA with probes for Ikaros exons 1 to 8. Results. Results of both gene expression and MLPA analysis were available for 182/244 children diagnosed between 2002 and 2007. MLPA revealed a deletion of at least one exon of IKZF1 in 12 (7%) patients. The proportion of non-DNA binding isoforms to the total IKZF1 expression was significantly increased (>80%) in 15 (7%) patients. The expression of a dominant-negative Ik6 isoform was significantly elevated (>50% of total) in 10 (6%) patients. Surprisingly, changes on DNA level were not always reflected in gene expression. Of 12 patients with gene deletion, only five had an increased short/long isoform ratio (4 pts due to Ik6 overexpression). The deletion on one allele did not cause a decrease in total IKZF1 gene expression level. Conversely, of 10 patients with Ik6 overexpression, six patients had no DNA alteration, suggesting a different mechanism of altered gene expression. Patients with IKZF1 gene deletion had significantly worse relapse-free survival (RFS) than other patients (5-year RFS 46±15% vs. 91±2%, p=0.0001). Patients with IKZF1 deletion had higher MRD in BM at day 33 (p=0.008). In gene expression analysis, Ik6 overexpression was the most important negative prognostic factor (5-year RFS 80±16% vs. 91±2%, p=0.0001), whereas elevated short/long isoform ratio (>80%) had only weaker impact (5-year RFS 69±18% vs. 90±2%, p=0.02). Gene expression of no other single IKZF1 isoform had impact on prognosis. Patients with IKZF1 deletion had higher MRD in BM at day 15 (p=0.009), day 33 (p=0.02) and at week 12 (p=0.01) and higher peripheral blood MRD at day 15 (p=0.05). Conclusion. Contrary to previous studies, we showed that deletions within IKZF1 locus do not necessarily correlate with altered Ikaros gene expression. Conversely, Ik6 overexpression was not accompanied by a deletion within IKZF1 locus in 60% of patients. Both DNA- and RNA-IKZF1 alterations had a strong negative prognostic impact in a cohort of children with BCR/ABL-negative ALL treated by a BFM-based protocol that did not use MRD in the risk group stratification. Ideally, both genomic and gene expression-based approach should be applied together for the evaluation of prognosis.

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Congenital neutropenia and host defence

1075

PLERIXAFOR IS A POTENTIAL THERAPY FOR MYELOKATHESIS, WHIM SYNDROME

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Background. Myelokathexis or WHIM syndrome (warts, hypogammaglobulinemia, immunodeficiency and myelokathexis) is a rare autosomal dominant disorder attributable to mutations in the CXCR4 gene. The WBC is usually <1.0 x 109/L with severe neutropenia and lymphocytopenia. Marrow examination shows abundant neutrophils with hypersegmented nuclei. The mutations in CXCR4 are activating and prevent the normal release of neutrophils from the marrow into the blood. Plerixafor is a small molecule inhibitor of the binding of CXCR4 to its ligand CXCL12. Subcutaneous administration of plerixafor causes a dose-dependent increase in circulating leukocytes. It increases circulating CD34+ cells and is currently used as an adjunct to granulocyte colony-stimulating factor (G-CSF) for mobilization of hematopoietic stem cells. Aim. Investigated the potential of plerixafor as therapy for myelokathexis/WHIM syndrome.

Methods. We enrolled 6 patients (4 female, 2 male, ages 28 to 73 years) in this study, with informed consent and institutional review board approval of the University of Washington and Federal approval for investigation use of plerixafor. Five patients from three different families had the same mutation (R334ter); the other patient had a novel mutation (S324F=S635ter). Single subcutaneous doses of plerixafor, increasing from 0.04 to 0.24 mg/kg, were administered at 2 to 4 day intervals. Complete blood counts were determined with an automated counter and leukocyte differential counts confirmed manually. CD34+ cells and lymphocyte subtypes were measured by FACS before and 6 hours after the 0.08 mg/kg dose. Plerixafor was discontinued if neutrophils were >2.0 x 109/L at 24 hours, after all doses were tested or if serious adverse events or illness occurred. Results were compared with five similarly studied normal subjects. Results. All 6 patients showed a prompt leukocytosis with maximum blood neutrophils and lymphocytes at 6 to 12 hours, declining toward baseline by 24 hours. Blood neutrophils peaked at 3.9 +/- 0.5 x 109/L (range 1.8 to 5.1 x 109/L) at 6 to 12 hours. Two of the 6 patients achieved > 2.0 x 109/L neutrophils at 24 hours. The lymphocyte responses were proportionally greater than the neutrophil responses. The greatest increase was in B cells (CD20+cells), a 60 fold increase at 0.08 mg/kg, CD34+ cells increased 6.8 fold at 0.08 mg/kg. Comparisons of patients and normal subjects showed larger proportional B and T cell responses in the patients. Hematocrit, hemoglobin and platelet counts of patients and normal subjects showed larger proportional B and T cell responses. Hematocrit, hemoglobin and platelet counts of patients and normal subjects showed larger proportional B and T cell responses. Hematocrit, hemoglobin and platelet counts of patients and normal subjects showed larger proportional B and T cell responses. Hematocrit, hemoglobin and platelet counts of patients and normal subjects showed larger proportional B and T cell responses. Hematocrit, hemoglobin and platelet counts of patients and normal subjects showed larger proportional B and T cell responses. Hematocrit, hemoglobin and platelet counts of patients and normal subjects showed larger proportional B and T cell responses.
Figure 1. Naive/memory T cell reconstitution post-transplant.

although they reacted vigorously when infused into congenic mice. Likewise, immune function of allogeneic doTC was not restored when pharmacologic GVHD prophylaxis with cyclosporine A (CSA) was administered post-transplant. Separation of GVH-reactions and transfer of immunity was possible by strict selection of TC subpopulations that are enriched for or contain exclusively MCMV-specific TC, such as memory (CD62L+CD44+) or M45-tetramer+CD8TC (CD8M45). HSC+CD8mem or +CD8M45 resulted in better survival and significantly stronger anti-viral responses in MCMV target organs (CD8mem: median 0.6% vs. CD8M45: 8.7% M45/hepatic CD8TC) than HSC+ToTC (>0.05% M45/CD8TC). Furthermore, transplantation of HSC alone or grafts composed of HSC+CD8TC subsets allowed the regeneration of a nascent doHSC-derived TC pool with naive and central memory TC, that could theoretically provide better protection against a broad range of future infections. In mice given HSC+ToTC >90% of TC were expanded to TC with an effector memory phenotype (Figure). Conclusions: 1) Recipients of HSC alone have stronger immune responses against MCMV compared to hosts given TC-replete grafts; 2) Residual host cells contribute substantially to protective immunity in recipients of HSC alone early pTx, but are eradicated by conventional TC-replete grafts; 3) GVHD-prophylaxis with CSA did not improve immune function; 4) HSC-derived TC arising in a healthy host are superior to those that develop and undergo selection in a GVHD-affected lymphoid system; and 5) Transfer of small numbers of highly selected cell subsets, such as tetramer-sorted MCMV-specific CD8 TC, is feasible, as these cells expand dramatically in a lymphopoenic environment and provide functional protection against infections. Our results challenge the conventional assumption that doTC in a hematopoietic allograft are required for optimal regeneration of the immune system. Rather, our studies suggest that long-term lymphoid function will greatly benefit from rigorous TC-depletion of the graft, and avoidance of GVHD.

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DIMINISHED LEVELS OF HAX1 OR ELA2 PROTEIN, BUT NOT MUTATED ELA2 PROTEIN LEAD TO DEFECTIVE MYELOID DIFFERENTIATION: IN VITRO MODEL OF CONGENITAL NEUTROPIA

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Severe congenital neutropenia (CN) is a heterogenous disorder of hematopoiesis characterized by a maturation arrest of granulopoiesis in bone marrow at the promyelocyte stage. CN is a multigene syndrome caused by e. g. inherent mutations in the elastase 2 (ELA2) gene or HAX1 gene. The pathomechanism of defective granulopoiesis in CN patients downstream of ELA2 or HAX1 mutations is not completely understood. It is also not clear, why the same ELA2 mutations lead to cyclic neutropenia (CyN) or CN. Mutations in the HAX1 gene led to absent HAX1 protein. Although it has been demonstrated that mutations in HAX1 could contribute to the apoptosis of myeloid cells due to defective mitochondrial membrane potential, definitive effects of HAX1 mutations leading to isolated ineffective granulopoiesis in CN are still not well understood. We aimed to analyse the effects of mutations in HAX1 or ELA2 genes on granulocytic differentiation in vitro. Intriguingly, in CD34+ granulocytic progenitor cells of CN patients harboring HAX1 mutations, G-CSF failed to upregulate mRNA levels of the HAX1 interaction partner, HCLS1, as compared to healthy individuals. We found that HCLS1 in a complex with HAX1 transduced the G-CSF signal via LEF-1 to the nucleus. We transduced the promyelocytic leukemia cell line NB4 with shRNA constructs specific for HAX1 or HCLS1. We also transduced NB4 cells with WT ELA2 cDNA or cDNA encoding mutated ELA2 (C425 and A145-152 mutations are CN-specific; S97L mutation is typical for both CN and CyN). We assessed myeloid differentiation of transduced cells by FACS analysis, RT-PCR and cell morphology. We found that inhibition of HAX1 or its interaction partner HCLS1 led to a significantly reduced differentiation of the NB4 promyelocytic cell line in response to ATRA (16.9 % of CD11b+ cells in HAX1 shRNA group, 20.1 % of CD11b+ cells in HAX1 shRNA group vs. 68.2 % of CD11b+ cells in ctrl shRNA group), which mirrors the situation in CN. However, mutated ELA2 did not affect myeloid differentiation and even lead to increased proliferative capacity of cells. Previously, we demonstrated that levels of ELA2 mRNA expression in myeloid progenitors as well as of plasma NE protein were markedly reduced in CN patients harboring mutations in either ELA2 or HAX1 genes, as compared to CyN patients and to healthy individuals due to a lack of LEF-1 expression (Skokowa et al. Blood 2009). Therefore, we analyzed if inhibition of ELA2 by ELA2-specific shRNA had any effects on myeloid differentiation. As a result, we found significant inhibition of ATRA-induced differentiation of the promyelocyte leukemia cell line NB4 after inhibition of ELA2, as compared to ctrl shRNA transduced cells (25.3 % of CD11b+ cells in ELA2 shRNA group vs. 41.2 % of CD11b+ cells in ctrl shRNA group). In line with diminished myeloid differentiation, mRNA expression of LEF-1 transcription factor was downregulated after inhibition of HAX1, HCLS1 or LEF-1. Taken together, diminished levels of HAX1, HCLS1 or ELA2 proteins lead to disturbed granulocyte differentiation. However, introduction of mutated ELA2 protein into cells has no effects on myeloid differentiation.

1079
UPDATE ON THE RISK OF SECONDARY LEUKEMIA IN GENETIC SUBGROUPS (ELA2, HAX1, WASP, G6PC3, P14) OF CONGENITAL NEUTROPIA IN EUROPE

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Conventional neutropenia (CN) is well known as one of the premalignant bone marrow failure syndromes with an overall incidence of secondary leukemias of more than 10 percent. With the identification of new causative gene mutations the number of genetic subgroups is still increasing. In this study we assessed the incidence and potential risk factors of leukemic transformation in CN patients with known gene mutations in ELANE, HAX1, G6PT, G6PC3, WAS, SBDS, TAZ1 and p14 or no identified mutation, respectively, by analyzing all available data from the European Branch of the Severe Chronic Neutropenia Registry (SCNR). For comparison we also analyzed patients with cyclic neutropenia (CyN) with or without ELANE mutations. Results from genetic testing were available for 195 of 311 CN patients. Of the 195 CN patients 63 patients revealed ELANE mutations, 25 HAX1 mutations, 46 SBDS, 18 WAS, 21 G6PT, 8 G6PC3, 4 p14 and 5 TAZ1 mutations. In addition, in 35 patients neither ELANE nor HAX1 mutation was detectable. 51 patients were not tested to date, but further genetic evaluation is not yet completed. Results from genetic testing were also available in 29 of 67 patients with CyN of whom 23 revealed ELANE mutations and 6 were negative for ELANE mutations. Secondary malignancies occurred in 37 of the 311 CN patients and 1 of the 67 patients with CyN. The distribution by genetic subtypes is shown in the table below.

Table 1.

<table>
<thead>
<tr>
<th>Gene Mutation</th>
<th>Patients (N)</th>
<th>MDS/Leukemia n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total CN</td>
<td>311</td>
<td>37 (11.9)</td>
</tr>
<tr>
<td>– ELANE-CN</td>
<td>68</td>
<td>11 (16.2)</td>
</tr>
<tr>
<td>– HAX1-CN</td>
<td>25</td>
<td>5 (20.0)</td>
</tr>
<tr>
<td>– ELANE neg/HAX1neg</td>
<td>35</td>
<td>6 (17.1)</td>
</tr>
<tr>
<td>– SBDS</td>
<td>46</td>
<td>4 (8.7)</td>
</tr>
<tr>
<td>– WAS</td>
<td>18</td>
<td>2 (11.1)</td>
</tr>
<tr>
<td>– G6PT</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>– G6PC3</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>– TAZ1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>– p14</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>– not tested</td>
<td>81</td>
<td>9 (11.1)</td>
</tr>
<tr>
<td>Total CyN</td>
<td>67</td>
<td>1 (0.2)</td>
</tr>
<tr>
<td>– ELANE-CyN</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>– ELANE neg-CyN</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>– not tested</td>
<td>38</td>
<td>1 (0.3)</td>
</tr>
</tbody>
</table>
All subgroups benefit from G-CSF treatment. However, patients requiring higher maintenance doses of G-CSF are at greater risk of leukemic transformation (Rosenberg, Zeidler et al., 2010). Conclusion. The incidence of secondary leukemia reflects the heterogeneity of congenital neutropenia ranging between no leukemia and 20 percent in patients with an underlying HAX1 mutation. However, patient numbers within each genetic subgroup are still limited. Patients with severe congenital neutropenia who have mutations in ELANE, HAX1, SBDS or WAS as well as those with no recognized mutation are at risk of secondary leukemia. Progression to MDS or leukemia has so far not been reported in G6PT, G6PC3, TAZ1 or p14 CN cases in our Registry. Despite mutations in the ELANE gene patients with cyclic neutropenia exhibit no increased risk for malignant transformation. Mutational analysis is helpful to identify the genetic cause of severe congenital or cyclic neutropenia but with limited numbers in genetic subgroups still does not serve to identify patients at risk of leukemic transformation.

**Stem cell transplantation - Clinical 3**

**1080**

**CD4+CD25+ REGULATORY T-CELL DEPLETION TO IMPROVE GRAFT-VERSUS-TUMOR EFFECT AFTER DONOR LYMPHOCYTES INFUSION: BIOLOGICAL PREDICTORS OF CLINICAL RESPONSE**

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Background. We very recently published the first clinical trial showing that regulatory T-cells (Treg) depletion can improve alloreactivity and likewise the GVT effect in humans in the setting of donor lymphocyte infusion (DLI) after HSCT (Maury et al. Sci Transl Med 2010). Here, we aim to analyze T-cell contents of donors and recipients before and sequentially after DLI to identify relevant biomarkers of clinical response. Methods. CD25+ Treg-depleted DLI (d-DLI) were given in 17 adult patients with malignancy relapse after HSCT. All but one had previously failed to respond to at least one standard DLI and none had experienced GVHD. Overall, GVHD induction through Treg depletion was obtained in 6 out of the 17 patients and associated with partial or complete remissions of hematological malignancies. With a median follow-up of 24 months after d-DLI, this group of patients (GVH+) had an improved survival (p=0.035) as compared to the 11 others without GVHD induced (GVH-). CD4+, CD8+, B and NK cell subsets were monitored using flow cytometry and FoxP3 expression assessed by RTqPCR on PBMC collected from recipients before and at 7 time-points during the first year after d-DLI. Results. The CD25 magnetic depletion led to a very high Treg-depletion rate in all d-DLI products (mean CD4+FoxP3+ cell-depletion rate of 98% in accordance with a FoxP3 expression decrease by 92% by RTqPCR) with no significant difference between GVH+ and GVH- patients. We could also not evidence any correlation between Treg monitoring in recipients after d-DLI and GVHD onset. However, a longitudinal monitoring of lymphoid subpopulations using a hierarchic clusterization (Genesis software, Graz, Austria) revealed that a lowering relative number of naive CD4+CD45RA+ cells among CD4+ T-cells over time correlated with GVHD induction. At day 15 after d-DLI, i.e. before the occurrence of any GVHD, a low level of naive CD4+CD45RA+ +/- CD62L+ cells correlated with GVHD onset (p=0.04, Fig 1). This remained significant at day 30 for both phenotypes of naive cells. Conclusion. The ability of Treg depletion to break immune tolerance in HSCT patients refractory to alloreactivity seems to be associated with the decrease of the naive CD4+ T-cell population in recipients. Such a polarization to a memory phenotype that we found associated with GVH/GVT effects might represent a new relevant parameter to improve and predict responses to Treg-based anti-tumor immunotherapies.

**1081**

**RELAPSE AFTER ALLOGENEIC HEMATOPOIETIC CELL TRANSPLANTATION FOLLOWING REDUCED INTENSITY CONDITIONING FOR AML AND MDS: DIAGNOSTIC AND THERAPEUTIC IMPLICATIONS FOR GRAFT CELLS**


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Relapse after allogeneic hematopoietic cell transplantation (HCT) following reduced-intensity-conditioning (RIC) in patients with AML and MDS remains a major hazard. Therefore, identifying predictive factors which allow successful prevention and management of relapse is still a major challenge. For this purpose, we analysed 256 consecutive patients (138 male/118 female); median age 62 years with AML, n=205 (80%) and MDS, n=51 (20%) who received HCT after 200 cGy TBI +
fludarabine 30 mg/m² for 3 days followed by mycophenolate mofetil and cyclosporine. Donors were matched unrelated (MUD) in 201 (78.5%) and matched related (MRD) in 55 (21.5%) patients. Disease-stage at HCT was complete remission (CR1), n=156 (61%), CR2,n=42 (16.4%), beyond CR2,n=57 (14.4%) and untreated-MDS, n=21 (8.2%). Cytogenticities were intermediate and high risk in 169 (66%) and 74 (29%) respectively. Relapse- incidence was 48%. Interestingly, disease-specific parameters such as diagnosis, disease-stage, high-risk cytogenticities, and initial leukemic CD34-phenotype had no impact on relapse. While the donor-associated factors graft-versus-host-disease (GvHD) and CD34-DCC day 28 strongly correlated with later relapse (p=0.0005). Irrespective of the seventy, patients with acute and/or chronic GVHD relapsed less frequently (15.6%) compared to 48% of patients without GVHD (p=0.0005). Irrespective of initial leukemic CD34-phenotype, CD34-DCC day 28 <90% was highly predictive of hematological relapse (HR) with 52% versus 50% relapse-risk if >90% (p=0.006). Only 4.5% of patients who relapsed had CD34-DCC <90%. Management of patients with (HR) and those with decreasing CD34-DCC below 90% without HR consisted of immunomodulation [tapering of immunosuppression and/or donor lymphocyte infusion] in 82.5%. Initial leukemic CD34-phenotype changed in 57% of patients at relapse with only 51% of patients having persistently positive CD34-DCC. Overall, 57% of patients with overt HR achieved CR and 47.5% of patients with declining CD34-DCC below 90% a sustained complete CD34-DCC. A negative leukemic CD34-phenotype at relapse (p=0.02), relapse beyond day 100 after HCT, therapy for a decreasing CD34-DCC, rather than a HR (p=0.05), and inducing GVHD with immunomodulation particularly in relapses within 100 days post-HCT (p=0.005) correlated with a superior response and survival after relapse. CR in the 51% of patients where GVHD was induced was 63% compared to 30% in patients without GVHD (p=0.004) highlighting the graft-versus-leukemia activity. After RIC-HCT, donor- rather than disease-related factors predict relapse. The 5-year disease-free survival and overall survival in AML and MDS. Interestingly, the initial leukemic CD34-phenotype, monitoring of CD34+-DCC is an excellent marker to identify patients at risk of relapse and guide early immunomodulation which effectively enhances the graft-versus-leukemia effect thereby preventing hematological relapses or successfully treating them. Nevertheless, further research is needed to optimize immunosuppressive regimens to maximize the graft-versus-leukemia effect without the injurious effects of graft-versus-host-disease particularly in the early post-HCT phase.

1082 IMPACT OF PROPHYLACTIC CD8-DEPLETED DONOR-LYMPHOCYTE INFUSIONS AFTER T-CELL DEPLETED ALLOGENIC HEMATOPOIETIC STEM CELL TRANSPLANTATION EM Wagner, D Wehler, K Kolbe, M Theobald, W Herr, R Meyer University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

The combination of reduced-intensity conditioning and *in vivo* T-cell depletion by alemtuzumab prior to hematopoietic stem cell transplantation (HSCT) has demonstrated efficient engraftment and reduced graft-versus-host disease (GVHD). However, this regimen is associated with slow lymphocyte recovery leading to a delayed anti-infectious and anti-malignant immunity. In a phase I study, we investigated the prophylactic use of CD8-depleted donor lymphocyte infusions (DLIs) to improve immune reconstitution. We have previously shown the feasibility of this approach and demonstrated that the CD8-depleted DLI reliably converted a decreasing T-cell chimerism (Meyer et al. Blood 2007 & BMT 2009), Here we provide the follow up data of 101 patients with different hematological malignancies with a median observation time of 1 year post HSCT (range, 1–80 months). The majority of patients either suffered from an acute leukemia / MDS (n=42), lymphoma (n=28), myeloma (n=17), or myeloproliferative neoplasms (n=12). The median age of the patients was 56 years (range, 20–71) and none of the patients had received conditioning regimens. 45 patients had undergone previous transplantations (autologous: n=43, allogeneic: n=2). The donors were matched siblings (n=15), matched unrelated (n=48), or unrelated donors with single HLA mismatches (n=38). The calcineurin-inhibitor used for GVHD-prophylaxis was intended to be tapered until day 50 post HSCT. In the absence of GVHD, CD8-depleted DLI were subsequently administered prophylactically in escalating doses starting with 1×10⁶ CD4 T-cells / kg bodyweight. Following this procedure, 59 patients received at least one dose of DLI. Among those patients who did not qualify for DLI, 46 patients had prior GVHD. In 16 patients, DLI were not administered for other reasons (donor unavailable, infections, relapse). In 64% DLI induced acute GVHD, which was the major reason for withholding the next DLI-dose step. The rate of acute GVHD > grade 2 was 30%. 10% suffered from extensive chronic GVHD. The 1 and 3 year overall survival was 65% and 45%, respectively. Survival significantly differed between the DLI and the non DLI group after 5 years (63% vs. 27%, p=0.002). Since this trial was not randomized, we also compared the DLI group to only those patients who did not receive DLI for other reasons than primary GVHD and found similar results (62% vs. 28%, p=0.01). Although DLI was associated with a survival benefit, the relapse rate did not differ from that of the non-DLI cohort. When we analyzed the diseases separately, there was no significant effect of DLI on the relapse rate, but we found a trend towards a lower relapse rate among AML/MDS- and delayed relapses in myeloma-patients. As expected, the presence of GVHD at any time was associated with a reduced relapse rate (55.8% vs. 80.5%, p=0.015). In summary, the prophylactic application of CD8-depleted DLI in the allo-HSCT was associated with a survival benefit. However, we were not able to relate this benefit to a decreased relapse rate. Our data strongly support a randomized trial, comparing prophylactic vs. preemptive / therapeutic DLI application in the context of T-cell depleted HSCT.

1083 SECONDARY MALIGNANCIES AFTER STEM-CELL TRANSPLANTATION IN THE ERA OF REDUCED-INTENSITY CONDITIONING; THE INCIDENCE IS NOT REDUCED A Shimoni, N Shem-Tov, Y Volchek, R Yerushalmi, A Nagler Chaim Sheba Medical Center, Tel-Hashomer, Israel

**Background.** Allogeneic stem-cell transplantation (SCT) is a potentially curative therapy for a variety of hematological malignancies and non-malignant diseases. Secondary malignancies are a known complication in long-term survivors. The incidence and kinetics of secondary malignancies have been reported mostly after myeloablative conditioning (MAC). Reduced-intensity conditioning (RIC) has been introduced over the last decade to allow SCT in patients not eligible for standard SCT. RIC has been shown to reduce the incidence of transplant-related complications, however due to the relative limited long-term follow-up of RIC recipients, the incidence and risk-factors for secondary malignancies following RIC have not been well defined. **Aims.** To determine the relative risks and risk-factors for secondary malignancies following RIC compared with MAC. **Methods.** A single institution database of 902 allogeneic SCTs given over the last 12 years was retrospectively reviewed to identify patients with secondary malignancies. Conditioning regimens included standard MAC (n=252), fludarabine-based RIC (n=452) or fludarabine-based reduced-toxicity myeloablative conditioning (RTC, n=198). The incidence of secondary malignancies was calculated by cumulative incidence analysis with death due to other causes considered competing risk. The incidence was correlated with patient and transplant characteristics. Three patients with PTLD and 2 with secondary leukemia and relapse of the prior malignancy after SCT were not considered having secondary malignancies in this analysis. **Results.** Twenty-two patients had secondary malignancies including squamous cell carcinoma of the skin (n=5), penis (n=1) vagina (n=1) and colorectal cancer (n=1), breast cancer (n=2), metastatic cancer of unknown primary (n=1), pancreatic cancer (n=1), metastatic sarcoma (n=1), Kaposi sarcoma (n=1) and donor-derived MDS/AML (n=3). The median age at SCT was 55 years (29-70). Nineteen patients were given fludarabine-based RIC/RTC and none had total-body irradiation. The median time from SCT to diagnosis of secondary malignancy was 38 months (7 months-11 years). Eighteen patients had prior chronic GVHD and 10 were still on immunosuppressive therapy at the time of diagnosis of secondary malignancy. The cumulative incidence of secondary malignancy 10 years after SCT was 5.5% (95% CI 3.9-9.1%). It was also higher in older patients (>50 years) than in younger patients (7.5% vs 4.4%, p=0.04). It was also higher in patients with a history of chronic GVHD; 11.2% Vs 2.1% (p=0.05). Secondary malignancies were less common in patients with CML and nonmalignant diseases compared with patients with prior chemotherapy; 1.1% and 6.5% (p=0.07). Patients given MAC had...
a cumulative incidence of 1.9%, compared with 7.8% for patients
given fludarabine-based RIC or RTC (p=0.02). Multivariable analysis
identified chronic GVHD and advanced age as adverse prognostic fac-
tors with hazard-ratios of 3.5 (p=0.03) and 2.9 (p=0.05), respectively.

Conclusions. Secondary malignancies are rare but significant complica-
tion after allogeneic SCT. Chronic GVHD and advanced age at SCT
predict for higher incidence. The incidence is not reduced in the RIC
era, possibly due to the inclusion of older and more heavily pretreated
patients to these protocols. The possible adverse effect of fludarabine
in the conditioning regimen cannot be ruled out. Larger studies with a
larger number of events are needed to confirm these observations.

EBV-ASSOCIATED POST-TRANSPLANT LYMPHOPROLIFERATIVE
DISEASE FOLLOWING ALEMTUZUMAB-BASED ALLOGENEIC
STEM CELL TRANSPLANT: CLINICOPATHOLOGICAL FEATURES
AND PREDICTORS OF OUTCOME

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Background. Epstein-Barr virus-associated post-transplant lymphopro-
liferative disease (EBV+PTLD) remains an important clinical problem
following allogeneic stem cell transplantation (alloSCT). In vivo T cell
depletion with Alemtuzumab has been previously thought to confer
only a small increase in PTLD risk and the characteristics and outcome
of such patients have not received detailed study. Furthermore, al-
though quantitative-PCR monitoring for EBV reactivation post-HSCT is
now commonplace, it’s diagnostic and predictive value remains unclear.

Aims. To characterise the clinicopathological features of PTLD occurring
after an Alemtuzumab-based alloSCT and describes a clinically diverse disease
that is often rapidly progressive. At onset of PTLD, EBV loads are fre-
cently below accepted thresholds for pre-emptive therapy, challenging
current paradigms for monitoring and intervention. Although a majority
of patients responded to Rituximab, 22% experienced progressive dis-
ease following immunochemotherapy. A 4-point score identifies those
predicted to have a poor outcome and for whom novel antibody thera-
pies or adoptive cellular therapy could be targeted.
**1085**

**A NOVEL SPLICED FUSION OF MLL WITH CT45A2 IN A PEDIATRIC BIPHENOTYPIC ACUTE LEUKAEMIA**

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**Background.** Abnormalities of 11q23 involving the MLL gene are found in approximately 10% of human leukemias. To date, nearly 100 different chromosome bands have been described in rearrangements involving 11q23 and 64 fusion genes have been cloned and characterized at the molecular level. In this work we present the identification of a novel MLL fusion partner in a pediatric patient with de novo biphenotypic acute leukemia. **Aims and Methods.** Cytogenetics, Fluorescence in situ hybridization (FISH), molecular studies (RT-PCR and LDI-PCR), and bioinformatics sequence analysis were used to characterize the CT45A2 gene as novel MLL fusion partner in pediatric acute leukemia. Results. Fluorescence in situ hybridization of the patient G-banded metaphases demonstrated a cryptic insertion of 11q23 in Xq26.3 involving the MLL gene. Breakpoint fusion analysis revealed that a DNA fragment of 653 kb from 11q23, containing MLL exons 1-9 in addition to 16 other 11q23 genes, was inserted into the upstream region of the CT45A2 gene located at Xq26.3. In addition, a deletion at Xq26.3 encompassing the 8’ region of the DDX26B gene (exons 9-16) and the entire CT45A1 gene was identified. RNA analysis revealed the presence of a novel MLL-CT45A2 fusion transcript in which the first 9 exons of the MLL gene were fused in-frame to exon 2 of the CT45A2 gene, resulting in a spliced MLL fusion transcript with an intact open reading frame. The resulting chimeric transcript predicts a fusion protein where the N-terminus of MLL is fused to the entire open reading frame of CT45A2. Finally, we demonstrate that all breakpoint regions are rich in long repetitive motifs, namely LINE/L1 and SINE/Alu sequences, but all breakpoints were exclusively identified outside these repetitive DNA sequences. **Conclusions.** We have identified CT45A2 as a novel spliced MLL fusion partner in a pediatric patient with de novo biphenotypic acute leukemia, as a result of a cryptic insertion of 11q23 in Xq26.3. Since CT45A2 is the first Cancer/Testis antigen family gene found fused with MLL in acute leukemia, future studies addressing its biologic relevance for leukemogenesis are warranted.

**1086**

**THE SINGLE NUCLEOTIDE POLYMORPHISM OF GENE XPA?XPC?XRCC1 AND IN ASSOCIATION WITH THE RISK OF ACUTE LYMPHOBlastic LEUKAEMIA**

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**Background.** Polymorphisms of DNA repair genes can alter protein structure and may impair DNA repair capacity. It is becoming clear that these polymorphisms may contribute to the risk of developing ALL. There are significant combinations between XPC Ala499Val and XPA A23G. Further studies are needed to elucidate potential functional relevance of the variant allele.

These results suggest that the XPA A23G and XPC Ala499Val polymorphisms may contribute to the risk of developing ALL. There are significant combinations between XPC Ala499Val and XPA A23G. Further studies are needed to elucidate potential functional relevance of the variant allele.

**1087**

**ACUTE LEUKEMIA IN CHILDREN WITH DOWN SYNDROME**

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**Background.** Down syndrome (DS) is the most common and the best known chromosomal disorder in human. The frequency is about 1 case in 800 live births and it is proportional with maternal age. The extra copy of the proximal part of 21q22.3 appears to result in the typical physical phenotype, mental retardation, hand anomalies, and congenital heart defects. Children with DS are predisposed to developing leukemia (1 case to 300 or 15-20 times more than in the general population). The frequency of acute lymphoblastic leukemia (ALL) to acute myeloid leukemia (AML) is 3:1. During the first 3 years of life AML is dominant, particularly transient myeloproliferative disorder (TMD) and acute megakaryocytic leukemia. Nearly all children with DS and leukemia have mutations in the hematopoietic transcription factor gene, GATA1. Children with DS-ALL are mostly of B-cell precursors origin and aberrant expression of cytokine receptor CRLF2, associated with mutated JAK2. **Aims.** To present our experience regarding to frequency, clinical features, treatment results and follow up of children with DS and leukemia. **Methods.** During the period from January 2006 to January 2011, 112 children with DS and leukemia was diagnosed. The frequency of DS is 1 to 1000 live births. Three children developed acute leukemia. Two boys, 1 and 2 years old, have AML (M0 and M7), one 3 years old has ALL and TMD was diagnosed in two newborns. First symptoms in all patients with acute leukemia were fatigue, petechial haemorrhagy, recurrent respiratory infection. Two newborns had conatal infection. Child with AML M7 is without cardiopathy. Child with AML M0 has VSD. The thirth with ALL has hypothyreosis, atriocentriculat septal defect and Morgagni hernia which both were corrected in the age of 3 months. One of newborns with TMD had Tetralogy of Fallot and the other VSD. Moderate splenomegaly was detected in all of the patients. Results. Laboratory data confirmed anemia (Hb range: 49-60g/l), WBC number from 4.7 to 74x10^9/l and severe thrombocytopenia (11 to 15x10^9/l). Bone marrow analyses confirmed M0, M7 and common ALL in three patients. Immunophenotype profiles are: patient with AML M0 had CD68 positive in 60% blast cells, CD79, CD20 and CD34 in 80%. Patient with AML M7 had expression of CD58, CD13, CD117, CD56, and CD34 and partial CD42b. Patient with cALL had BCR-ABL1 negative, MLL and TCF3 rearrangement negative, ETV6-RUNX1 positive. CD19, CD34, CD22, CD79A positive. Children with AML were treated according ML DS-BFM 2006 protocol and they are in complete remission 2 and 2.5 years after completed therapy. Newborn with complex cardiopathy died six month later during serious pulmonary infection but with normal blood and bone marrow features on 1 month age. The other is still alive without any sign of TMD. **Conclusions.** Children who develop AML have generally favourable prognosis. Outcome of DS-ALL has been considered worse than in a ratio of ALL without DS. Majority DS-ALL may benefit from therapy blocking the CRLF2/JAK2 pathway and for DS-AML, mutations of GATA1 gene.

**1088**

**IMMUNOGLOBULIN AND T-CELL RECEPTOR GENE REARRANGEMENT PATTERN IN CHILDHOOD ACUTE LYMPHOBlastic LEUKAEMIA WITH MLL GENE REARRANGEMENTS**

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**Background.** Rearrangements of the mixed lineage leukemia (MLL) gene are the most common of all gene rearrangements found in infant acute lymphoblastic leukemia, occurring in approximately 15% of patients younger than 1 year. Although less frequently, the MLL gene is also rearranged in older children (≥1 year), particularly in CD10-negative ALL.
cases. These three factors: MLL rearrangements, younger age and pro-B immunophenotype are inter-correlated and closely associated with poor prognosis. They have also been postulated to influence the immunoglobulin (ig) and T-cell receptor (TCR) gene rearrangement pattern. Aims. We aimed at characterization of Ig/TCR gene rearrangement pattern in a group of 19 MLL-positive ALL patients: 12 infants (10 pro-B-ALL, 2 b-cell precursor ALL with myeloid co-expression) and 7 patients older than 1 year, aged 1.6-17.5; mean age: 7.8, median age 6.5 (6 pro-B-ALL and 1 common/pro-B-ALL). We also aimed at comparison of the Ig/TCR pattern identified in the study group with the pattern previously reported in a group of 58 B-cell precursor ALL (BCP-ALL) patients. Methods. Polymerase chain reaction with the use of standard BIOMED-1 and BIOMED-2 primers was used for detection of Ig/TCR gene rearrangements. Clonality of the rearrangements was assessed by heteroduplex analysis and confirmed by sequencing. Results. At least one clonal rearrangement was found in 68% of 19 MLL positive patients, in contrast to 97% in 58 BCP-ALL patients. The frequencies of rearrangements in the MLL positive group were as follows: 32% VH-(DH)-JH, 26% DH-JH, 52% total IGH, 11% VK Kde, 21% Vβ2-Dβ3, 11% Vy, 5% Dβ1β2 vs. 74%, 9%, 74%, 31%, 45%, 50%, 4%, respectively, in MLL positive group (DH-JH and Dβ1β2 rearrangements were studied in a subgroup of 25 BCP-ALL patients). In contrast to BCP-ALL group no Intron-Kde, Dβ2-Dβ3, Vβ2-Dβ3αβ2β and Vβ-Dbβ2β rearrangements were found in the MLL positive patients. Summary. As compared to BCP-ALL group Intron-Dβ2β and total IGH rearrangements were characterised by more immature pattern (i.e. rearrangements are less frequent with predominance of IGH rearrangements, relatively frequent occurrence of DH-JH rearrangements and lower frequencies of IGH-Kde and cross lineage TCRD, TCRG, TCRB rearrangements; complete TCRB and TCRD/A rearrangements were not found in the study group). The study will be continued in a larger cohort of MLL positive patients (68%).

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1089 DNA METHYLATION PATTERN IS ALTERED IN CHILDCHOOD T-ALL AS COMPARED TO T CELL SUBSETS AND HEALTHY CHILDREN

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Summary. The therapy success rate for T-ALL is lower than in precursor B-ALL, which reflects higher T-ALL aggressiveness and its heterogeneous biology. In many cancer subtypes it is possible to distinguish heterogenous DNA hypermethylation patterns. Identification of patient-specific CpG island methylator phenotype (CIMP) might facilitate treatment stratification. Aim. We aimed at assessing CIMP patterns in T-ALL Patients and Methods. Methylation status of 20 genes was assessed by MS-PCR in 63 children with de novo, T-ALL treated at the centres of Polish Pediatric Leukemia and Lymphoma Study Group (PPLLSG). Additionally, 11 healthy bone marrow donors younger than 17 years of age and 15 healthy children from control group were tested as controls. Results. Two groups of patients were delineated: CIMP- and CIMP+. Additionally, computational clustering of patients was concordant with CIMP groups (p<0.0001). Analysis of correlation between CIMP and traditional clinical risk assessment factors, EGIL-T-ALL classification or NOTCH1 and FBXW7 mutation status, showed no significant association. Conclusion. Methylation pattern is different in T-ALL patients. It is possible to divide patients into two groups characterized by two main methylation patterns. Results of our study indicate existence of CIMP phenomenon in childhood T-ALL, although its biological and prognostic significance needs further studies.

1090 CDKN1A-MEDIATED RESPONSIVENESS OF ACUTE LYMPHOBLASTIC LEUKEMIA TO AURORA KINASE INHIBITORS

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Background. The prognosis of acute lymphoblastic leukemia (ALL) is related to age and cytogenetic status. Unfavorable cytogenetic abnormalities and old age in ALL will lead to dismal outcomes. Recent studies showed that Aurora kinases were therapeutic targets in cancer therapy, including acute myeloid leukemia, Philadelphia-positive leukemia and multiple myeloma. It is currently unclear whether the therapeutic activity of the compounds in leukemia is primarily due to selective Aurora kinase or multi-kinase inhibition. Aim. The aim of this research was to investigate the molecular mechanism regulating the differences in the responsiveness of ALL to Aurora kinase inhibitors. Methods. In this study, we used Aurora kinase inhibitor "VE-465". Nine ALL cell lines containing t(4;11) and non-t(4;11) ALL cell lines were used to evaluate the expressions of Aurora kinases by Western blot and RT-PCR and the treatment effect of Aurora kinase inhibitors by MTT assay. The effects of Aurora kinase inhibitors on the cell cycle were evaluated by flow cytometry. The expressions of CDKN1A (p21) in mRNA and protein levels were compared between the drug-sensitive and drug-resistant cell lines. Results. Cells treated with Aurora kinase inhibitors (VE-465) inhibited the phosphorylation of Aurora kinase effectively. Aromatase negative pine needle ALL cell lines, RS4;11 was more sensitive to Aurora kinase inhibitors (IC50<10 nM) and the treatment resulted in an increased G2/M and sub-G1 populations. RPMI-8402 and Raji were most resistant to Aurora kinase inhibitors. Expression of Aurora kinases and their activators did not correlate with the drug susceptibility in ALL cell lines. Treatment effect of Aurora kinase inhibitors in ALL cell lines. Further investigation of the role of CDKN1A in the response to Aurora kinase inhibitors is warranted in the future.

1091 EFFECTS OF FORMIN-LIKE 1 (FMNL1) SILENCING IN A HUMAN LYMPHOBLASTOID CELL LINE

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Background. FMNL1 has a highly restricted expression in malignant lymphoid derived cells, including in cells from chronic lymphocytic leukemia and Non-Hodgkin lymphoma patients; and has been described
as a tumor associated antigen. The function and regulation of FMNL1 have yet not been well characterized; however its restricted expression suggests that FMNL1 represents an attractive target for novel immunotherapies in hematopoietic malignant disorders. Aims: Herein, we evaluated the role of FMNL1 in proliferation, colony formation and migration in the Namalwa cell line, a human B lymphoma cell line. Methods: Specific shRNA-expressing lentiviral vector targeting FMNL1 or LacZ were used. Cells were used. Cell growth, x-gal analysis, migration and colony formation were measured using MTT colorimetric reduction method. Colony formation was carried out in semisolid methyl cellulose medium and was detected after 8 days of culture by adding 1mg/mL of MTT reagent and scored by Image J quantification software. Both assays were carried out in lentiviral transfected cells, treated or not with different concentrations of rapamycin (10 or 100nM). Migration assays were performed in triplicate using 5-μm Transwells and the lower compartment was filled with 600 μL 0.5% BSA/RPMI containing 100ng/mL SDF-1. P value <0.05 was considered statistically significant. Results: The levels of FMNL1 mRNA and protein in FMNL1 knockdown cells were reduced by approximately 70%. Inhibition of FMNL1 resulted in a significant decrease of proliferation and clonogenicity by 40% and 32% respectively, when compared with control cells (P<0.001). Interestingly, the combination of FMNL1 inhibition/rapamycin treatment showed higher reduction in both assays when compared with FMNL1 inhibited cells alone (P<0.01) or rapamycin treated cells (P<0.05). Moreover, FMNL1 silencing resulted in a significant decrease of cell migration rate (P<0.01). Conclusions: Our findings indicate that FMNL1 participates in the regulation of proliferation, colony formation and migration of the Namalwa cell line, which suggests that FMNL1 represents an attractive therapeutic target. Interestingly, we observed a synergistic effect on FMNL1 silencing and rapamycin inhibition in proliferation and clonogenicity suggesting that they may act through different pathways. Based on this result, we hypothesized that combinatorial inhibition of these pathways would be effective for the treatment of lymphoid malignancies. Supported by FAPESP, CNPq and Instituto Nacional de Ciência e Tecnologia do Sangue-INCT do Sangue.

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AUTOMATED ANALYSIS OF CEREBROSPINAL FLUID (CSF)
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Background. Reference method for CSF cells count is cytometric chamber nowadays. However this method is quite slow; inter-observer variability has been reported and some errors are commonly committed. So, automated and standardized methods are desirable to simplify and to reach reliable results in the analysis. On the other hand, early detection of CSF neoplastic cells is very important in order to establish a suitable treatment in cancer diseases. New hematologic autoanalyzers with bioinformatics incorporated can help to detect neoplastic and non neoplastic cells. Sysmex XE-5000 TM (SXE) are capable to carry out quantitative and qualitative analysis. We also compared SXE CSF results with those obtained by cytospin microscopic observation and flow cytometric studies. Methods. 232 CSF samples from patients with hematologic- ic diseases (acute leukemia and NHL n=216), non hematologic cancer diseases (n=4) and infectious, inflammatory diseases and others (n=12) were analysed. CSF samples were firstly acquired in SXE counter and then manually processed for cytospin, May-Gründwald-Giemsa stain and microscopic observation. Flow cytometric analysis (FC) with four colour monoclonal antibodies according to pathology was also carried out. When initial diagnosis was unknown we used HLA-DRFITC / CD19PE / CD3PECy7/ CD4APC panel. All events from 0.5-1 ml sample were acquired (BD FACSCalibur) and analyzed (BD machine). Cell growth - x-gal analysis was used. Samples were classified as follows: non cellular, neoplastic or activated. Results. Sensibility for leukocytes detection was higher in SXE than cytospin interpretation (88% versus 51% respectively). 42.9% of neoplastic infiltrated samples showed more than zero HFBF, but only 8% of non cellular and 10% of activated CSF samples. We found statistical differences in HBF percentages between all groups (Kruskal Wallis p<0.001). When we applied U de Mann-Whitney test, we could find higher HBF % in infiltrated samples compared to non cellular (p<0.001) and to activated ones (p=0.015). Differences in HBF% between activated and non cellular samples (p=0.001) were not found. Infiltrated samples by FC had a tendency to have higher HBF values. However, within the three classified types it was more frequent to find values close to zero. Conclusions. SXE autoanalyser could be a rapid, reliable and straightforward method to detect and differentiate SCF cells. High HBF values in neoplastic samples might suggest a valid screening tool when flow cytometry is not available.

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MIR-155 INHIBITION EFFECT ON CELL PROLIFERATION AND APOPTOSIS INDUCTION OF JURKAT (ACUTE T CELL LEUKEMIA) CELL LINE
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Backgrounds. MicroRNAs are small non-coding RNA molecules with approximately 22 nt in length and cause inhibition of translation or degradation of mRNA. Mir-155 is a molecule with different functions, such as role in proliferation and immunity. Overexpression of this miRNA has been found in a number of cancers. One of its best known functions is apoptosis in case of caspase-3 activity. Aims. The main aim of this study was evaluation of LNA mir-155 inhibitor effect in apoptosis. Methods. In this study, Jurkat cells were used and for evaluation of sensitivity to varied concentrations (25, 50 and 75 nmol) of mir-155 inhibitor using MTT assay. Mir-155 expression level was analyzed using the quantitative real-time polymerase chain reaction (QRT-PCR). Caspase-3 activity was measured by caspase-3 colorimetric activity assay kit. Unpaired t test test were used for analysis of the MTT and apoptosis results. Probability of 5% was assumed as statistically significant. Results. According to our results, the use of mir-155 inhibitor increased activity of caspase-3 by 2 fold in 75 nmol concentration. In this research, we found that the proper increase of mir-155 inhibition can inhibit mir-155 and consequently increase caspase-3 activity and induce apoptosis in the Jurkat cells leading to cell death ultimately. Conclusions. Apoptosis stimulation by miRNAs is probably one of the best and low risk way of cell death induction in malignancies. Due to role of mir-155 in several cancer cells, it may be used as a therapeutic tool in future.
chemotherapy), decrease that correlate more frequently with an increase in total body fat, hypertriglyceridemia, hyper-LDL-cholesterolemia, hypo-HDL-cholesterolemia, a more frequent increase of fasting insulinemia, and a more frequent hypertension. In the study of a group of patients treated with both chemotherapy and radiotherapy, where the MS was present in a proportion of 53.33%, after 12 months of treatment with GH, the MS was present in only 5.56% of them, and after 24 months in none of them had MS; they also noted a decrease in left ventricular mass index and an improvement of cardiac systolic function. The cardiovascular toxicity of chemotherapy consists especially in left ventricular dysfunction induced by anthracyclines and in endothelial dys- function produced by the increase of homocysteine levels induced by methotrexate. Summary. The criteria that define MS are frequently present in patients that were anterior treated for ALL. Anterior cranial radiotherapy favors a decrease in GH levels (more than chemotherapy), decrease that correlate more frequently with the presence of MS compounds. There is the necessity of studies that compare the metabolic dysfunctions present before ALL with those that appear after the treatment.

1095

ADOLESCENT AND YOUNG ADULT ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEDIATRIC PROTOCOL: A TUNISIAN MONOCENTRIC STUDY

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Background. Pediatric protocols give good results in childhood acute lymphoblastic leukemia (ALL) comparing to adults treated with other protocol. Aims. We report in this study the results of pediatric protocol used for adolescent and young adult acute lymphoblastic leukemia (ALL). Method. Between January 2000 and December 2007, we retrospectively analyze the data of acute lymphoblastic leukemia patients aged from 16 to 30 years treated according to the pediatric EORTC 58951 protocol. Patients were stratified in average and high risk groups according to white blood count at diagnosis, blasts phenotype, cytogenticities abnormalities and response to corticotheraphy (cortico-sensitivity: blasts less than 1000/mm3 at day 9) and chemotherapy (complete remission: CR). Finally we analyzed the 5 years overall survival (OS), event free survival (EFS) and disease free survival (DFS). Results. Thirty three adolescent and young adult were treated according to the EPRTC 58951 pediatric protocol. Median age was 18 years (16 to 28) and sex ratio was 1.53. Median WBC was 52,000/mm3. B and T phenotype were observed in respectively 38% and 62% cases. Cortico-sensitivity was noted in 73% of patients. Three patient dead during induction and eight dead after, sep-sis was the frequent cause of mortality (10 cases). Twenty nine patients achieved CR (96%). Four patients relapsed. Five years OS, EFS and DFS were respectively 48, 48 and 79%. Conclusion. This study showed that pediatric protocols give good results concerning CR and DFS to adolescents and young adult ALL. However OS an EFS, better than adult ALL treated during the same period by adult protocol (OS=18%, EFS=18% and DFS=24%) was not yet satisfactory because the high toxic mortality rate.

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ANTIOXIDANT STATUS IN EGYPTIAN CHILDREN WITH MALIGNANCIES

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Background. Combination of chemotherapy received by Children with malignancies makes them prone to peroxidative injury. Objectives. The aim of this study was to evaluate antioxidant status in children suffering from different types of malignancies and to estimate the relation between antioxidants status and chemotherapy related side effects. Patients and methods. In a sample of 60 children having different childhood malignancies, antioxidant status was evaluated initially at diagnosis, after 3 and 6 months (6 ms) of therapy by measuring endogenous antioxidants (uric acid, albumin, bilirubin), exogenous antioxidants (vitamin C, total antioxidant capacity (TAC)), and Malondialdehyde as oxidative marker. Results. Regarding exogenous antioxidants there was no statistical significant changes in albumin, bilirubin and uric acid, after 3or 6ms of therapy. Vitamin C and TAC showed significant reduction during follow up period when compared to baseline levels (p<0.001 for all) while Malondialdehyde levels showed significant increase after 3 and 6 ms of chemotherapy when compared to baseline levels (p<0.001). Marked decrease level of endogenous antioxidants over 6 ms of chemotherapy treatment was associated with dose reduction and increase incidence of induction (p=0.001, p=0.002 respectively) but there was no significant changes in stoppage of chemotherapy and hospitalization throughout treatment when compared to baseline rate. No correlation between VIT C, TAC, and Malondialdehyde levels were found (r=0.17, P=0.37, r=0.09 p=0.63, r=0.00 p=0.99). Levels of exogenous antioxidants and Malondialdehyde were not different among hematological and solid type of malignancies or in different age groups and both sexes. Patients with solid tumors showed significantly higher rate of chemotherapy stoppage after 6 ms compared to hematological tumors (p=0.05). Conclusion. Children undergoing treatment for malignancy receive combination of chemotherapy which is associated with free radical production and increase oxidative stress in those children and related to complications of treatment.

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HOW TO TREAT ADOLESCENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Adolescents with acute lymphoblastic leukemia (ALL) have languished in the shadow of successful therapeutic outcome in childhood ALL. While 80% of children aged 1-15 years are long-term survivors, less than 40% of adults are cured with current therapies. Adolescents and young adults who may be eligible for both adult and pediatric protocols have continued to have an intermediate outcome, which has remained inferior to that in children. Aims. This article has summarized the recent and updated retrospective comparative analysis of adolescents treated with pediatric and adult trials. Methods. Data on ALL adolescents aged 16-18 treated with pediatric trial ALL IC-BFM 2002 were compared with literature reports on the treatment of adolescents according to adult protocols. Results. During the 2002-2006 period, 145 ALL patients aged 16-18 were treated with pediatric trial ALL IC-BFM 2002. The 3-year event free survival (EFS) was 71%. During the 1997-2002 period, 67 ALL patients aged 15-17 were treated with adult trial UKALL XII/E2993, and 61 ALL age-matched patients were treated with pediatric trial MRC ALL 97, with 3-year EFS of 49% and 65%, respectively. A similar 3-year EFS has been reported for the American pediatric regimen (CCG) that was by 26% higher in comparison with their adult regimen (Cancer and Leukemia Group B, CALGB). Conclusion. Therapeutic outcome in adolescents with ALL treated according to pediatric protocols is better (16 - 26%) in comparison with adult regimen.

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LONG-TERM OUTCOME AND PROGNOSTIC IMPACT RISK FACTORS IN 117 ADULT ACUTE LYMPHOBLASTIC LEUKEMIA PATIENTS TREATED WITH CHEMOTHERAPY WITH OR WITHOUT STEM CELL TRANSPLANTATION

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Background. Adult acute lymphoblastic leukemia (ALL) is often incur-
able despite intensive chemotherapy with or without hematopoietic stem cell transplantation (HSCT). Estimated overall long-term survival rate for adult ALL patients (pts) is about 20-50%. Aim. To present our results of treatment adult ALL and impact high risk factors on overall survival (OS). Patients and Methods. Since January 1989 till January 2011 we were treated 117 (male 85, female 45) pts with adult ALL, average age 30 years. Ph-like type metaphase cytogenetic was determined in 85 (B-ALL 57, T-ALL 28) and cytogenetic/ molecular analysis were succeeded in 55 pts. At the time of initial presentation: 29 pts had high white blood cell (WBC), 5 pts had CNS involvement and 19 pts had mediastinal mass. Pts treated with induction, consolidation and maintenance therapy under modified FAB regimen for high risk (82), HyperCVD (13), LALA 94 (6), CHOP, BFM, GMALL, EJOG UKCR, etc. (14). High risk factors were defined by the presence of at least one of following factors: age > 35 years, WBC > 30x10^9/l (B-ALL) or 100x10^9/l (T-ALL); CNS involvement, more than 4 weeks to achieve complete remission and finding Philadelphia chromosome (or bcr-abl+), t(4;11)+ or t(1;19)+. Medicament prevention (without radiotherapy) of CNS disease was applied to every pts under 50 years. Results. Complete remission (CR) was achieved in 104 pts (delayed CR in 18). Resistant to therapy were 5 (4.8%) pts; 6 (5%) pts have died (2 early deaths, 2 before evaluation of remission, and 2 in CR during the intensification due to infective complications). Maintenance therapy (MT) was applied for 24-26 months. HSCT was done in 44 pts: allo in 22 pts (CR1 in 15, CR2 in 8, and with partial response in 2 pts), auto in 17 pts (CR1 11, CR2 5 and with molecular relapse 1 pts). Relapses have occurred in 67 pts (63%) with median time of 9 months. Frequency of relapses were higher in pts on MT (45/62 - 73%) comparing to HSCT pts (22/44 - 50%); auto HSCT 10/17 - 58% and allo HSCT 12/27 - 44%, respectively. The secondary allo HSCT was done in 5 pts (2 are still alive). Long-term survival without relapses had 19/106 (27.4%) pts; 10-years disease free survival (DSF) in MT pts was 13.65 +/- 5%, (2 are still alive). Long-term survival without relapses had 19/106. With IFI. Our results suggest that continued low dose intermittent antifungal strategy during this phase of treatment has yet to be established although is of importance given the significant risks associated with fungal infections through subsequent consolidation treatment during which itraconazole prophylaxis appears ineffective. The optimal antifungal prophylaxis in adult patients undergoing ALL induction. We therefore applied this regimen to our TYA population receiving treatment according to the UKALL 2003 protocol. 20 consecutive patients (aged 16-24) with newly diagnosed disease treated at a single centre (March 2007 - Jan 2011) were included in this retrospective series. 3 were non-evaluable as they received induction treatment elsewhere. Chemotherapy consisted of induction (55 days) comprising weekly vincristine (x5) and daunorubicin (x4), daily dexamethasone and 2 doses of pegylated asparaginase (days 4 and 18) followed by standard BFM consolidation comprising weekly cytarabine (4 days per week for 16 doses), daily 6-mercaptopurine and 2 doses of cyclophosphamide (days 1 and 15). 2 patients received augmented BFM consolidation. Patients were hospitalised during induction during which time they received antifungal prophylaxis using Amphotericin. 17 patients received Abelcet (100mg 3 x per week by intravenous infusion). Only one patient was intolerant of Abelcet and was therefore treated with Ambisome (5mg/kg once weekly by intravenous infusion). Consolidation chemotherapy was predominantly administered in the clinic and upon completion of vincristine, antifungal prophylaxis was switched to daily itraconazole liquid (200mg bd) or capsules (200mg tds). Fungal infections were categorised into possible, probable or proven according to the EORTC/MSG criteria. Four patients developed a possible or proven IFI during the study period with a cumulative incidence of 32.5% (95% CI 16.4-64.2%). All infections occurred during the consolidation phase with one death due to infection. A further 5 patients received empiric antifungal therapy (1 during induction and 2 during consolidation) due to suspected fungal infection (predominantly unresponsive fever) with no evidence to meet the EORTC/MSG criteria for a possible IFI. Our data indicate that low dose Abelcet is an effective and well-tolerated strategy for primary antifungal prophylaxis in TYA patients undergoing induction chemotherapy for newly diagnosed ALL. However, patients remain at high risk of developing fungal infections following subsequent consolidation treatment during which itraconazole prophylaxis appears ineffective. The optimal antifungal prophylaxis in adults undergoing induction chemotherapy for newly diagnosed ALL is therefore very helpful.

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LOW DOSE AMPHOTERICIN LIPID COMPLEX FOR PRIMARY ANTI FUNGAL PROPHYLAXIS IN YOUNG ADULT PATIENTS UNDERGOING INDUCTION CHEMOTHERAPY FOR ACUTE LYMPHOBLASTIC LEUKAEMIA

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Patients with acute lymphoblastic leukaemia (ALL) are at high risk of developing invasive fungal infections (IFI) during induction treatment with significant mortality and morbidity. The reported incidence of IFI is up to 40% and with an associated reported mortality of 20-50%. In Teenage and Young Adult (TYA) patients, there is increasing use of pae- niostatin-based protocols, however the increased intensity of these regimens potentially increases the risk of developing IFI. There is no consensus regarding the optimal antifungal strategy in this population. In addition the interaction between vincristine and azoles restricts the choice of drugs. We have previously reported the efficacy and tolerability of low dose amphotericin B lipid complex (Abelcet) as anti-fungal prophylaxis in adult patients undergoing ALL induction. We therefore applied this regimen to our TYA population receiving treatment according to the UKALL 2003 protocol. 20 consecutive patients (aged 16-24) with newly diagnosed disease treated at a single centre (March 2007 - Jan 2011) were included in this retrospective series. 3 were non-evaluable as they received induction treatment elsewhere. Chemotherapy consisted of induction (55 days) comprising weekly vincristine (x5) and daunorubicin (x4), daily dexamethasone and 2 doses of pegylated asparaginase (days 4 and 18) followed by standard BFM consolidation comprising weekly cytarabine (4 days per week for 16 doses), daily 6-mercaptopurine and 2 doses of cyclophosphamide (days 1 and 15). 2 patients received augmented BFM consolidation. Patients were hospitalised during induction during which time they received antifungal prophylaxis using Amphotericin. 17 patients received Abelcet (100mg 3 x per week by intravenous infusion). Only one patient was intolerant of Abelcet and was therefore treated with Ambisome (5mg/kg once weekly by intravenous infusion). Consolidation chemotherapy was predominantly administered in the clinic and upon completion of vincristine, antifungal prophylaxis was switched to daily itraconazole liquid (200mg bd) or capsules (200mg tds). Fungal infections were categorised into possible, probable or proven according to the EORTC/MSG criteria. Four patients developed a possible or proven IFI during the study period with a cumulative incidence of 32.5% (95% CI 16.4-64.2%). All infections occurred during the consolidation phase with one death due to infection. A further 5 patients received empiric antifungal therapy (1 during induction and 2 during consolidation) due to suspected fungal infection (predominantly unresponsive fever) with no evidence to meet the EORTC/MSG criteria for a possible IFI. Our data indicate that low dose Abelcet is an effective and well-tolerated strategy for primary antifungal prophylaxis in TYA patients undergoing induction chemotherapy for newly diagnosed ALL. However, patients remain at high risk of developing fungal infections following subsequent consolidation treatment during which itraconazole prophylaxis appears ineffective. The optimal antifungal prophylaxis during this phase of treatment has yet to be established although is of importance given the significant risks associated with IFI. Our results suggest that continued low dose intermittent amphotericin B lipid complex may be effective although is inconvenient in the outpatient setting.
1101 THE OUTCOME OF THE ADULT PH-POSITIVE ACUTE LYMPHOBlastic LEUKEMIA PATIENCE FROM AN EAST EUROPEAN COUNTRY IN TKIS ERA - THE EXPERIENCE OF ROMANIAN WORKING GROUP FOR ADULT ACUTE LYMPHOBLASTIC LEUKEMIA (RWGAL)

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Background. Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (Ph+ALL) includes at least one-quarter of all adults with ALL. The conventional chemotherapy programs that are effective in other precursor B-cell ALL cases are unable to cure these patients. The availability of imatinib mesylate and other tyrosine kinase inhibitors (TKIs) changed the treatment programs and the prognosis for these patients. In Romania imatinib combined with chemotherapy was approved as standard therapy for Ph+ALL in 2006. We studied the outcome correlated with treatment programs for 21 patients with novo Ph+ALL. Materials and methods: There were 11 male and 10 female, aged between 17 and 71 years (median = 48y) diagnosed 2007-2009. Immunophenotypic diagnosis was ALL preB for 9 patients, ALL - common for 10 and biphenotypic acute leukemia for 2 patients. All patients (even at 71 years old) received curative chemotherapy for induction and consolidation and imatinib, 600 mg daily (except 1 patient who received 400mg/d). Results. Complete remission achieved 76% patients at on average of 44.8 days from diagnosis (limits 24 - 66 days). Complete molecular response was documented for 42.5% patients, at a median of 365 days (limits 177 - 465 days). Imatinib was started after remission induction and/or after first consolidation. For 57.14% of patients chemotherapy during imatinib was low dose or reduced intensity. Due to SCT policy in our country for ALL, no patient was transplanted in first remission. PFS was 13.8 months (limits 3 - 57 months). Relapsed treatment was: intensive chemotherapy for 4 patients, 1 patient switch to dasatinib and other 3 patients received tandem therapy with dasatinib and nilotinib. Only one patient was transplanted in the second CR, and received Dasatinib after transplant. At the end of our study, 5 patients were living at 8, 14, 16, 20 and 37 months respectively, 4 of them with CR, one relapsed. Disease/relapse related mortality was 27.7%. Treatment related mortality was 44.4%. Overall survival was on average 12.25 months.

Conclusions. The prognosis of adult patients with Ph+ ALL treated only with chemotherapy is poor, with a less than 10% probability of long-term survival. The use of imatinib as part of front-line treatment in combination with cytotoxic agents has greatly improved the rates of complete hematologic and molecular remission and overall outcome in adult patients with newly diagnosed Ph+ ALL. However the allogeneic WBC is the only treatment option with definite curative potential. Our data show that only aggressive chemotherapy combined with imatinib would really change the outcome of Ph+ALL new diagnosed patients.

1102 EVALUATION OF THE CCG 1991 AND CCG 1961 PROTOCOLS OF THERAPY OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA IN TWO EGYPTIAN ONCOLOGY CENTERS: A PILOT STUDY

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Clinical trials in childhood ALL have yielded 5-year event-free survival (EFS) rates as high as 80%, yet we did not reach comparable results partly due the high mortality and morbidity rates associated with high dose methotrexate therapy. Since 2004, the Pediatric Oncology Centers in Ain Shams and Menoufeya University shifted the protocol of therapy of standard risk ALL to the CCG 1991, and the high risk ALL patients to the CCG 1961. The aim of this study was to evaluate the efficacy of the CCG protocols (CCG 1991 and CCG 1961) in the treatment of childhood ALL in Ain Shams and Menoufeya University Pediatric Oncology Centers and the associated morbidity and mortality rates.

Methods: A prospective, multicenter, including 191 patients 2-18 years old diagnosed in both University Centers in the period from April 1st 2004 to December 31st 2005, recruited after having parental consent. Patients were classified into 3 risk groups, standard risk (SR), high risk standard arm (HR-SA) and high risk augmented arm (HR-AA) based on clinical and morphological data and response to therapy. Risk stratification used did not include minimal residual disease. Protocol CCG 1991 (Arm OS with single delayed intensification) was used for standard risk patients. Protocol CCG 1961 (Arm A standard BFM arm of standard duration) was used for HR-SA patients, and CCG 1961 (augmented BFM arm using doxorubicin) was used to treat HR-AA acute lymphoblastic leukemia (Ph+ALL). The mean age was 5.9 (+3.3) years, male to female ratio was 1.61. CNS leukemia was present in 6% of patients at diagnosis. 14 patients (26.9%) had T-ALL and 38 (73.1%) had preB-ALL. Initial total leukocytosis count >50,000/static was present in 16 patients (30.8%). According to risk stratification: 25 patients were SR (48.1%), 16 HR-SA (30.8%), and 9 HR-AA (17.3%). The 5-year overall survival (OS) and 5-year EFS of the total number of patients was 83% and 67% respectively. The 5-year overall survival (OS) was 97%, 55%, 88%, for SR, HR-SA, and HR-AA, respectively. The 5-year EFS for the SR, HR-SA and HR-AA patients was 60%, 58% and 70% respectively. Grade 3-4 adverse events were reported in five patients (10%). Relapse and death occurred in 3(12%) and 1(4%) in SR patients respectively in (21.5%) and 4(30.8%) in HR-SA, and in 1(8.3%) and 1(8.3%) in HR-AA respectively (P=0.05). Conclusion: The outcome of SR and HR-SA protocols used was not satisfactory, accordingly intensification of protocols was done shifting SR ALL to CCG 1961 arm OD (double delayed intensification), and shifting HR-SA to CCG 1991 HR-SA ARM B (standard BFM arm) and DDI. Cytogenetics and Minimal residual disease assessment are mandatory for prediction of risky patients in SR ALL and in HR-SA groups for better risk stratification of therapy to achieve better childhood ALL survival.

1103 CHEMOTHERAPY RELATED ACUTE SIDE EFFECTS IN CHILDREN TREATED FOR ALL

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Background. The survival rate in childhood ALL has reached to 80% with the contemporary chemotherapy protocols and increased supportive care. However, chemotherapy related acute side effects may still cause serious problems leading to morbidity and mortality during treatment. Although the reports about the late effects among the survivors are frequent, the data related to the acute adverse effects are scarce. Aims. This study evaluated the acute side effects of chemotherapy in children with ALL. Method. The acute toxicity according to the WHO criteria for each chemotherapy phase in 210 children treated as ALL between the years 1993 and 2009 were, retrospectively, evaluated from their hospital records. Children received either BFM-95 (76.5%) or BFM-2000 (%23.5) protocols. Results. The data of 187 children were evaluable. The mean age and male to female ratio were 6.2±1.7m and 1.7, respectively. The median follow-up period was 61±43.4m. Children were stratified into 3 risk groups as standart (19.8%), median (61.5%) and high (18.7%). General well being was similarly effected during the induction phase in all risk groups (p<0.05). However, poor condition was significantly more frequent in high risk group blocks (p<0.001). Bone marrow involvement was more prominent during induction phase in each risk group, however, it was significantly depressed during high risk blocks (p<0.05). The frequency of nausea were found similar during all treatment phases. Vomiting, stomatitis, diarrhea were significantly occurred in high risk blocks along with elevated liver transaminases (p<0.001). MTX related effects were observed in 3.2% (n:6) of the patients. Allergic drug reactions due to L-asparaginase including urticaria and/or ed skin changes (Grade 1/2) were observed in 3.2% (n:6) of the patients. Vomiting, stomatitis, diarrhea were significantly occurred in high risk blocks (p<0.05). The frequency of nausea were found similar during all treatment phases. Vomiting, stomatitis, diarrhea were significantly occurred in high risk blocks along with elevated liver transaminases (p<0.001). MTX related effects were observed in 3.2% (n:6) of the patients. Allergic drug reactions due to L-asparaginase including urticaria and/or ed skin changes (Grade 1/2) were observed in 3.2% (n:6) of the patients. Vomiting, stomatitis, diarrhea were significantly occurred in high risk blocks (p<0.05).
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GASTROINTESTINAL TOXICITY SECONDARY TO TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: The putative tumor suppressor gene called CDKN2/INK4A/p16 was also mapped to chromosome band 9p21. The gene codes for cyclin-dependent kinase inhibitors (CDKI). These CDKIs can bind and inhibit the kinase function of the cyclin-dependent kinase 4 (CDK4) and CDK6, resulting in the blockade of the cell cycle between G1 and S, leading to a suppression of cellular proliferation. For this reason, these CDKIs may act as tumor suppressors. Therefore, their inactivation might contribute to development of cancer. Deletion of the 9p21 chromosomal region are frequent in childhood acute lymphoblastic leukemia (ALL) but, the prognostic significance is controversial. In childhood ALL, CDKN2/INK4A/p16 inactivation is found in more than 20% of B-lineage ALL and 50% of T-ALL. Methods: We studied 85 children (aged 12 months to 17 years) consecutively diagnosed as ALL. Of the 85 children, 64 had B-ALL, whereas 21 had T-lineage ALL. All patients were treated according to the protocols of the BFM-ALL 2000 between January 2008 and December 2010. CDKN2A inactivation by deletion was studied using Fluorescence In Situ Hybridization (FISH). Results: Bi-allelic and mono-allelic deletion were found in, respectively, 4 (4.7%) and 8 (10.5%) of 84 children. At the time of analysis, the median follow-up was 1.2 years (range 6 months-3 years). Summary: A pathogenic role for p16 gene deletion in the development or progression of those ALL, suspected because of the potentially antiproliferative properties of p16, will have to be clearly shown.

Table 1. Clinical outcome according to 9p21 status

<table>
<thead>
<tr>
<th>9p21 Status</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>56.2%</td>
</tr>
<tr>
<td>Deletion</td>
<td>30.7%</td>
</tr>
<tr>
<td>Other</td>
<td>13.1%</td>
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PEDIATRIC-LIKE INTENSIFIED THERAPY IN ADULT ACUTE LYMPHOBLASTIC LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background. Acute lymphoblastic leukemia (ALL) shows different outcomes in children and adults with event-free-survival (EFS) rates of 70-80% and 30-40% at 5 years, respectively. Recently, results improved in young adults/adolescents aged 15-21, with de novo ALL, when treated with pediatric intensive regimens rather than with typical adult regimens. Clinical studies are ongoing in older patients, toxicity related-therapy seeming the limiting issue. Aims. We report a single centre experience on intensive ALL patients treated with an intensive pediatric inspired schedule, aiming to assess its tolerability and efficacy. Methods. From 11/07 to 02/11 we treated 22 ALL patients (M/F=16/6) according to modified AIEOP-LAL2000 regimen. Treatment consisted of 7 days steroid pre-treatment, and four drugs 78-days induction (phase IA and IB) after which high risk (HR) patients were treated with three polychemotherapy blocks, while intermediate (IR) and standard risk (SR) patients went on 8-weeks consolidation and subsequent intensification. A 2 cycle consolidation therapy with nelarabine was planned for T-ALL patients. Patients with HLA-matched donor underwent allo-SCT; 2-years maintenance therapy was given to the others. Median age was 42 years (17-70); 20/22 patients completed the phase IA, 2 being out for grade IV toxicity (intestinal occlusion and sepsis), 15 (68%) obtained a complete remission (CR), 5 (23%) were refractory. Three of the resistant patients subsequently achieved CR: one after polychemotherapy blocks, two after phase IB. Median induction duration (IA+IB) was 95 days (52-136), delays were mostly accumulated during the interval between phase IA and IB and rare due to logistic reasons and extra-hematologic toxicity. A higher absolute number of adverse events during phase IA than during IB was registered (infections and gastrointestinal), without a significant prolongation of phase IA. After induction, 3 of the 15 CR patients received consolidation therapy, then 2/3 underwent allo-SCT. 5 patients received blocks under allo-SCT, 2/5 dropped out after the first and the second block for reversible grade II-III renal toxicity. 3 patients were treated with nelarabine, then 1/3 underwent allo-SCT. 3/5 directly underwent allo-SCT, while 1 patient completed the whole

GASTROINTESTINAL TOXICITY SECONDARY TO TREATMENT OF PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Background. Acute lymphoblastic leukemia (ALL) is the most frequent cancer in childhood. Although intensive chemotherapy has improved survival in those patients, important side effects, including acute gastrointestinal (GI) toxicity are frequent. Although GI toxicity is frequently described in reported ALL series of patients, the different criteria used to define severe GI toxicity translates in very different incidence of such complications in the literature. Aim. To describe the incidence of severe acute GI toxicity in a series of ALL patients treated in a single centre during the last 11 years, and to report the short and long-term outcome of these complications. Patients and methods. Data from children diagnosed with ALL in Hospital Sant Joan de Déu from July 1999 to January 2011 were collected. Patients were treated according to two sequential trials of the Pediatric Hematology and Oncology Spanish Group (SEHOP), SHOP ALL-99 and SHOP ALL-2005 that include standard agents for ALL treatment. Severe (grade III-IV) GI complications were analyzed. Results. During this period 145 children were diagnosed with ALL at our institution. Sixteen of them (11.1%) presented enterocolitis-lymphitis and two (1.3%) gastroenterocolitis during the induction phase (n=101) and intensification (n=1). The main clinical findings were abdominal pain (100%), defined as severe in 77% of cases, and mucositis (77%). Neutropenia was present in 83% of the patients (severe neutropenia in 55%), with a mean duration of 15 days. Blood cultures were positive in 4 children for S. aureus, P. aeruginosa, E. coli and S. epidermidis, respectively. Most patients (n=10) improved with medical treatment but 5 patients (5.4%) needed surgical treatment and one child died because of refractory sepsis (P. Aeruginosa). Twelve patients (8.3%) presented an elevation of pancreatic enzymes (amylase ranged from 114 to 1499 U/L and lipase from 158 to 455 U/L), related to L-asparaginase administration. Seven patients were asymptomatic and the rise in pancreatic enzyme was transient; however, five patients (3.4%) presented an acute pancreatitis with severe abdominal pain that needed analgesic treatment and delayed the scheduled chemotherapy administration. One patient died from fulminant pancreatitis and the other patients had a favorable outcome. Three patients (2%) presented a grade III-IV increase in bilirubin during induction phase. One of them presented a fulminant hepatitis and died, while the others improved after adjustment of the chemotherapy doses. Overall, the incidence of severe GI toxicity in our series was 16% and the mortality rate due to GI complications was 2%, similarly to other previously reported data. Conclusions. GI toxicity of standard chemotherapy regimens is frequent in ALL patients. Close attention of pancreatic enzymes is necessary during the L-asparaginase administration, but in most cases isolated rise in pancreatic enzyme levels in an otherwise asymptomatic patient will be transient and will not require treatment interruption. Although most GI complications were manageable with medical treatment, some patients eventually died due to GI toxicity.
therapeutic program because no suitable donors for allo-SCT were found. Median CR duration was 12 months (9–44); 6 patients relapsed, 3/6 after allo-SCT. With a median follow up of 13 months (3–49), 14/22 (65.6%) patients are alive, 7 in CR (4 underwent allo-SCT). 8 patients died, 4 for relapsed/refractory disease, 4 in CR (3 after allo-SCT). On the basis of pediatric management, minimal residual disease assessments on days 55 and 76 of induction were scheduled. Important technical and logistic requirements were formulated. BCDR was performed only for 7 of the 15 CR patients, due to no probe individuation, no sample collection or degraded sample at diagnosis. Conclusions. A pediatric-inspired therapeutic regimen seemed to be feasible in adult ALL patients, even if significant delays due to logistic reasons and extra-hematological toxicities were under control in maintenance. Therefore an effort to collect and analyze data from this population should be made in order to offer the best therapeutic options available.

1108
EXPRESSION OF PIM-2 AND NF-KB IS INCREASED IN PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML) AND ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AND CORRELATES WITH COMPLETE REMISSION RATE
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Background. PIM-2 is a proto-oncogene that encodes for serine/threonine kinase which interacts with various signalling molecules. PIM-2 is highly expressed in neoplastic tissues and in leukemic and lymphoma cell lines which is consistent with a its role during oncogenic transformation. The nuclear factor kappa B (NF-kB) pathway appears to be deregulated in variety of tumors, with sustained activity of NF-kB leading to apoptotic resistance in tumor cells. The aim. The aim of this study was to investigate whether the PIM-2 and NF-kB expression is altered in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Patients and methods. One hundred forty-three patients were included: 91 with AML and 52 with ALL (42 with B-ALL and 10 with T-ALL), aged 18–84 (median–41). Seventy-five patients reached complete remission (CR): 50 in AML and 25 in ALL. Bone marrow samples were collected at the time of diagnosis. Control samples were obtained from 24 healthy donors. We analysed PIM-2 and NF-kB expression by RQ-PCR analysis. Results. Expression of both PIM-2 and NF-kB in all leukemic patients and in subgroups: AML and ALL was significantly higher than in controls. In AML, PIM-2 was expressed in 85% of patients with CR at significantly lower levels than patients with primary resistance to chemotherapy (with no CR, NCR). Moreover in AML, we have found the correlation between PIM-2, NF-kB expression and blasts in myelogram and PIM-2 and patients’ age. Summary. Our data indicate that PIM-2 and NF-kB genes expression is increased in patients with AML and ALL and correlates with CR rate in AML patients.

1109
DYNAMICS IN SOME PARAMETERS OF IRON STATUS MARKERS AND ANTIOXIDANT SYSTEM IN PATIENTS WITH ACUTE MYELOID LEUKEMIA
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Background. Iron overload is a negative marker in patients with myeloid neoplasms as it associated with non-leukemic events including VOID, GVHD and fungi infection. The mechanism of tissue damage by free iron includes formation of NTBI and LPI with following excess generation of ROS. Intensive chemotheraphy is possible way of NTBI formation in patients with AML and risk factor of infection in collaboration with immunosuppressive therapy. The aim. To find out the association between dynamics in some parameters of iron status and AO system in AML patients after intensive chemotheraphy. Methods. The serum of 14 AML patients with median age 46 y (34–68) was prepared before and after chemotherapy, at day of WBC level >1x10⁹/L and day of the following hospitalization. The chemotherapy regimens were 7+3 induction therapy (3 patients), courses with Ara-C dose <1 g/m² (8 patients) and Bu+Cph before AutoSCT (2 patients) and AlloSCT (1 patient). The levels of transferrin saturation, malondialdehyde concentration, and activity of superoxide dismutase, catalase and ceruloplasmine were detected by standards assays. Results. After chemotherapy the range of TS levels was 92.8%–97.1% (p=0.021). Activity of catalase was significantly lower before chemotherapy: 200.8% vs 158.7%; p=0.001. At the time of hematopoiesis restoration the level of TS was lower than after chemotherapy: 89.5% vs 96.8%; p=0.003. Activity of antioxidant enzymes was changed differently, and the variability of MDA levels during and after chemotherapy was without any significant change. After chemotherapy and during the period of aplasia the activity of catalasa was significantly lower than before therapy: 3.8 and 3.5 vs 5.7; p=0.028 and p=0.011. Activity of SOD was decreased only at the time when WBC was ≥1.0x10⁹/L: 21.0 vs 41.0; p=0.018. At the same time the activity of CP was significantly higher than before chemotherapy: 1.1 vs 0.8 g/L; p=0.029. Three episodes if infection complications were registered after chemotherapy. One patient was diagnosed with herpes simplex skin involvement after restoration of hematopoiesis. Two cases of sepsis were diagnosed during aplasia with one lethal outcome. Summary. As the level of TS exceeded ≥80% is associated with NTBI formation. Sahlstedt L. et al. Br.J.Haematol
we conclude that there is a phase of free iron overload in AML patients during the period of post-T cytopenia. The data support the idea that the changes of AOS are the compensatory mechanism which have to deteriorate the complications of non-transferrin-bound iron after chemotherapy. The prospective clinical trial will be design to compare the clinical effectiveness of antioxidants after intensive chemotherapy.

### 1110

**METABOLISM OF MEMBRANE PHOSPHOLIPIDS IN ACUTE LEUKEMIA AND DURING OF CHEMOTHERAPY**

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**Background and aims.** Membranes of blood lymphocytes were charac-
terized with the significant phylogenetically stabilized phospholipids-phospholipids interrelations. It is well known that abnormalities of these interrelations play an important negative role in the development of path-
ogenic mechanisms, which condition inactivation of the membrane bound enzymatic systems, catalyzing the reactions transportation through membranes, as well as transduction of the external signals into the cell. The quantities and qualitative structure of phospholipids in the lymphocyte membranes, as well as lipids peroxidation processes and activity of phospholipase A2 have been studied in patients with acute leukemia and during of chemotherapy. Materials and methods. Experimen-
tal studies were carried out on 24 patients with first diagnosed acute leukemia. Biochemical analyses were done on first day and on tenth day: one group during traditional chemotherapy, second group during chemotheraphy with additional antioxidant therapy. Fractionation of the individual phospholipids (PL) was realized by thin-layer chromatogra-
phy with silica gel LC 5:40 m. After that the lipid phospholipids was defined. Lipids peroxidation activity has been determined by the reaction of malonic dialdehyde with thiobarbituric acid by the known method. Study of phospholipasa A2 activity was accomplished by spectropho-
etric assay method. Results and conclusions. Our investigation showed to acute leukemia it is peculiar essential disturbance of the qualitative and quantitative contents of almost all representatives of lymphocytes membrane PL, mainly phosphatidylcholines. Under these conditions a pro-
nounced increase of cytoytic lysosphosphatidylcholones (LPC) is accom-
ppanied by simultaneous pronounced of phosphatidylcholines (PC) con-
centration, which testifies about phospholipase A2 and lipid peroxidation processes activation. Hence, the acute leukemia is characterized by noticeable increase of the contents of diphosphatidylglycerides (DPG), while spingomyelins (SM), phosphatidylinositols (PI), phosphatidyl-
ethanolamine (PE) and phosphatidylserine (PS) decrease. While using chemotherapy alone in the treatment of acute leukemia is usually pro-
grammed for and followed by certain positive clinical outcomes, the com-
parison of such treatment with additional antioxidant therapy results in more expressed biochemical changes. This method is bringing to nor-
malization of phospholipase A2 and lipid peroxidation activity and bal-
anced qualitative and quantitative structure of lymphocyte membrane PL. The changes of membrane PL can be qualitative characteristics of process activity. The same time level of balancing qualitative and quantitative structure of lymphocyte membrane PL is vulnerable index to appreciate treatment effectiveness.

### 1111

**TRIPSTATE: A NEW ACUTE MYELOBLASTIC LEUKEMIA MARKER**

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**Background.** Triptase is the main protein of human mast cell secreto-
ry granules. Recent studies have shown that serum triptase levels are high in 40% of patients with acute myeloblastic leukemia (AML), es-
pecially in those with favourable karyotype, invit 16 and (t6,21), with or without association of KIT mutations. Aims. To study the incidence of high triptase levels in acute myeloblastic leukemia. To analyze the cor-
relation between high triptase levels and other markers like CBF alter-
ations and KIT mutations. To analyze its relevance as minimal residual disease (MRD) biological marker. Methods. The study was performed prospecively in prospectively on a total of 58 patients with acute myeloblastic leukemia (except promyelocytic M3): 29 men and 19 women (27-87 years old). Serum triptase levels were quantified by FEIA. Acute myeloblastic leukemia subtype, karyotype and molecular biolo-
gy were examined at diagnosis of every patient included. Results. Out of 58 patients, 17 showed high triptase levels (29%); 1 M0, 1 M1, 4 M2 (1 M2 Eo), 6 M4 (5 M4 Eo), 2 M5 and 3 unknown subtype. Taking into account molecular alteration, high triptase levels were observed in 3 of 4 AML-ETO positive patients (one of them with KIT mutation), of 1 of 2 patients with inv16 and 2 of 9 patients with FLT3-ITD mutations. Higher triptase levels (>200) were observed associated with inv 16 and (t6,21). Out of 45 patients without 7 patients with high triptase level had a con-
tentation data of 12. We observed that out of these 12 patients, 6 reached normal triptase levels concurring with complete remission of the disease and in 2 patients, high triptase level kept despite achieving CR (these patients had the highest triptase levels among all the samples processed, 165 and > 200) and levels came back to a normal range afterwards. Out of these 12 patients, 4 showed refractory disease; 3 of them kept high triptase lev-
els and in the one left (with slightly high levels at diagnosis), triptase came back early to a normal range, despite having active disease. From the patients who achieved CR, just one relapsed. She has a central nerv-
ous system relapse and bone marrow relapse late in time, showing an increase in triptase levels with the bone marrow relapse. Conclusions. 29% of AML patients showed high serum triptase levels, which is lower than the incidence published so far. The higher triptase levels (> 200 µL/L) were found in patients with AML-ET0 and inv 16, there seems to be an association with CBF rearrangement, as it has been described before. Triptase levels tend to decrease when CR is achieved, so it might be useful as a MRD biological marker, taking into account that should be confirmed studying a greater number of patients.

### 1112

**PLATELET AGGREGATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES -CORRELATIONS WITH THE FLUIDITY MEMBRANE CHANGES AND ROS LEVEL**

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**Background.** Patients with myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) present severe alterations of platelet func-
tion. Platelets from these patients are dysplastic and have alterations in membrane and granular content. Aim The purpose of this study was to identify abnormalities in platelet function and a possible correlation with changes in platelet membrane fluidity and the reactive species level. Material and method We present a prospective study on 75 cases with AML or MDS compared with 55 cases with AML admitted in University Emergency Hospital Bucharest. Three patients with AML were investigated in early stages and the phase of complete remission of the disease. Platelet function was investigated by platelet aggregation using as stim-
uli ADP, collagen, epinephrine and ristocetin. Membrane fluidity was assessed by fluorescence anisotropy measurements using TMA-DPH, the ROS level determination was performed using DCFDA method. Results. Platelet aggregation was altered in both groups of patients, more pronounced for AML patients. (AML vs. MDS: ADP 28.10 vs. 37.88, p=0.009; collagen 40.69 vs. 58.37, p=0.008; epinephrine vs. 16.48 24.16, p=0.14; ristocetin 43.73 vs. 50, p=0.37). No significant differences were obtained in the lag phase in the two groups of patients. The platelet aggregation response was improved in remission phase of AML for all reagents. The membrane anisotropy was increased in AML patients compared with MDS patients( r = 0.1711 vs. 0.1372, p = 0.002), this result correspond to low fluidity of membrane. There was not obtained a statistically significant correlation between the degree of membrane anisotropy and severe platelet function. Reactive species level is slight-
ly higher in AML patients (0.0005280 vs. 0.0004051, p = 0.52). This lev-
el is significantly increased in advanced phase of disease. (LAM patients 0.0006078 vs. 0.0001413, p = 0.003; MDS patients 0.0005452 vs. 0.0001804, p=0.009) we could not establish a correlation of the fluidi-
ty changes depending on the level of ROS. Conclusion. AML patients have an advanced degree of alteration of platelet function and a low flu-
dity of platelet membranes compared to MDS patients. Achieving a clinical remission may improve platelet function, possibly due to a more appropriate expression of platelet receptors and / or improving cellular signaling, possibly correlated with low levels of reactive species.
1113

BENZENE - THE MOST IMPORTANT LEUKEMOGENIC FACTOR IN VOJVODINA

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Background. Benzene is the most toxic substance for hematopoesis. Hematologic effects are expressed as cytopenia, aplastic anemia, myelodysplasia, acute leukemia and other hemoblastoses, due to genotoxic effect or epigenetic modification. The most common consequence is acute myeloblastic leukemia (AML). There is no safe benzene concentration, no tolerance to benzene, and susceptibility is individual. Permissible concentration of benzene does not exist. Risk of AML is increased at cumulative exposure above 2 ppm-years and with intensity over 0.8 ppm. Inhalation is the most common exposure to benzene. During benzene metabolism, produced free oxygen radicals damage DNA, which could cause gene mutation or epigenetic modification with gene silencing. In Vojvodina, with petrochemical industry, agriculture with high pesticide usage and intensive road traffic, permanent increasing trend of AML was recorded during the last three decades. On the basis of our clinical studies this can be explained by higher exposure to benzene. Of all adult patients with AML in Vojvodina 25% are from Novi Sad. Aims. The aim of this study was to analyze consequences of benzene exposure in residents of Novi Sad who are living near oil refinery. The second aim was to evaluate the potential genotoxic damage. The following points remain of occupational exposure to benzene. Methods. Oxidative stress (OS) and DNA damage in the group of 60 residents environmentally exposed to benzene was investigated measuring 8-OHdG, which implies oxidation of DNA, in urine by gas chromatography-tandem mass spectrometry (GC-MS). Control group represented 60 residents from other parts of Novi Sad. Genotoxic biomarkers were investigated in peripheral blood lymphocytes in 313 refinery workers exposed to petroleum and its derivates using sensitive sister chromatid exchange test (SCE) and in minority of the workers by mononucleus test (MN). Control group represented persons without contact with benzene. Results. Examination of OS in environmentally exposed residents showed increased values of 8-OHdG. This result was significantly higher than in control group. Genotoxic biomarkers SCE and MN were significantly higher (p<0.05) in workers directly exposed to benzene in comparison to office workers. Cigarette smoking additionally increased SCE frequency. Conclusions. These results confirm our previous clinical studies which implicated that benzene is the most important leukemogenic factor. Low doses of benzene after inhalation can be enlarged by additional dermal contact or oral benzene intake with water. It is possible that low doses of benzene act in synergism with confounders (smoking, radiation, chemicals). Low doses of benzene and its metabolites could cause epigenetic modifications and immunodeficiency of the basis of our research we would strongly support regulations for benzene concentration in Serbia and reduction of occupational full shift exposure in the range of 0.5-1 ppm, without high short exposures. Adequate individual protection, monitoring of biomarkers and hematologic examination are necessary. For ambiental air benzene concentration European limit is 5µg/m3 and benzene in gasoline is low (<1%). In agriculture biopesticides and biofertilizers are recommended. Persons with signs of oxygen stress, positive biomarkers for genotoxicity or epigenetic modification need chemoprevention by dietary polyphenols. Some AML patients with epigenetic modifications could be treated with hypomethylating agents.

1114

ACUTE MYELOID LEUKEMIA WITH INV(3)(q21q26.2) OR T(3;3)(q21;q26.2): IMMUNOPHENOTYPIC CHARACTERISTICS IN 35 PATIENTS FROM A SPANISH RETROSPECTIVE MULTICENTRIC SURVEY

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6Hospital Germans Trias i Pujol, Badalona, Spain
7Hospital Clinic i Provincial, Barcelona, Spain
8Hospital Vall d’Hebron, Barcelona, Spain
9Hospital Doctor Trueta, Girona, Spain
10Hospital de Fuenlabrada, Madrid, Spain
11Hospital Doce de Octubre, Madrid, Spain
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14Background. The 2008 WHO classification recognizes acute myeloid leukemia (AML) with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) as an independent clinicopathological entity, with an aggressive course and short survival. Its incidence is very low (1% of all AML) and immunophenotypic studies of this type of AML are limited. Aims. The purpose of the study was to analyze retrospectively the immunophenotypic features of leukemic blasts in patients with inv(3)(q21q26.2) or t(3;3)(q21;q26.2), through collecting a significant number of cases within the Spanish Group of Hematological Cytology (a working group into the Spanish Hematology and Hemotherapy Society). Methods. We collected a total of 35 cases (25 patients with AML inv(3) and 12 with AML t(3;3), mean age 50 years, range 14-84, males 54%), diagnosed between 1983 and 2010, in 13 national hospitals. During the time they were diagnosed, 2-, 3- or 4-color flow cytometric analysis was performed on peripheral blood or bone marrow aspirate specimens collected in EDTA. Although the panels of MoAb were not exactly similar in different centers, all included the most frequently used at diagnosis in acute leukemias. Cases were considered positive if 20% or more of the cells expressed the specific antigen. We compare our findings with those reported by Medeiros et al. in the most important series published to date (Leuk Res, 2010). Results. The following table compares the findings in our series with those published by Medeiros et al.

Table 1. NE: not evaluated.

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<th>MoAb</th>
<th>Medeiros et al. (N = 15)</th>
<th>Spanish Group of Hematological Cytology (N = 35)</th>
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<tr>
<td>CD34</td>
<td>13/15 (87%)</td>
<td>27/28 (93%)</td>
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<td>CD38</td>
<td>15/18 (83%)</td>
<td>26/28 (93%)</td>
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<tr>
<td>CD41</td>
<td>5/15 (33%)</td>
<td>11/28 (39%)</td>
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<tr>
<td>CD45</td>
<td>15/15 (100%)</td>
<td>27/28 (93%)</td>
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<tr>
<td>CD56</td>
<td>14/15 (93%)</td>
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In our experience, more than 80% of the patients were positive for CD34, CD38, CD11b, CD117, and HLA-Dr. Of note, contrary to Medeiros et al. and similarly to the revised 2008 WHO classification, we find a considerable high proportion of cases showing CD38 positive blasts. But also a relatively high rate of patients (>40%) were positive for CD123, CD11b, CD34 or CD15. The aberrant expression of CD4 is found in more than one third of cases. In our study, positive expression for CD3, CD8, CD10, CD20, TdT, or glycophorin (all antigens not analyzed in other study) was never observed. Conclusions. Basically our results are consistent with those found by Medeiros et al., and blasts exhibit an immature myeloid phenotype with a frequent aberrant expression of CD7. However, an expanded panel can also find cases

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positive for CD123, CD11b, CD36, CD15 and CD4. For the best of our knowledge, this is the largest series of patients with AML with inv(3)/t(3;3) that specifically studies the immunophenotypic characteristics of leukemic blasts.

1115
THE CHLOROQUEINE DIPHOSPHATE CHANGES PROTEIN PNAS-2'S SUBCELLULAR LOCATION IN LEUKEMIC CELLS AND INDUCE THEM TO APOPTOSIS

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Background. Previous studies in our laboratory had found PNAS-2 was an anti-apoptosis gene and might participated in leukemogenesis, this was confirmed by scholars in Germany. When we studied Pnas-2-GFP fusion protein, we found Pnas-2's subcellular location in leukemic cells might be abnormal with unstable membrane of multivesicular body. Some articles had reported chloroquine diphosphate (CQ) could stabilize membranal structures. 

Aims. We hypothesized that CQ might normalize Pnas-2's subcellular location in leukemic cells and by this way induce them to apoptosis. In this study, we explored whether CQ could induce leukemic cells to apoptosis or stabilize membrane of multivesicular body in leukemic cells. 

Methods. Both leukemic cell lines such as HL60, U937 and leukemia primary cells were studied. All samples were obtained with informed consent. De novo samples of 12 patients with acute leukemia (AL) were used in this study, including 1 M3, 6 M4, 5 M5 and 1 ALL patients. Seven samples of relapsed acute myeloid leukemia (1 M2, 4 M4 and 2 M5) and 4 CR patients (1 M5, 2 M4, 1 M5) were also assessed. Immunofluorescence method were applied to investigate Pnas-2's subcellular location by using monoclonal antibody of PNAS-2 which was prepared by our laboratory. Annexin-V-APC/PI kit was used, after preparation of cells, membranes were stained with Annexin-V, and cytosolic DNA was stained with PI. The flow cytometer was used to analyze the apoptosis rate.

Results. We found just like we suspected previously, Pnas-2 protein was not only located in vesicles nearby the nucleus but also distributed throughout the cytosol in leukemic cells, instead of in vesicles which was nearby the nucleus in non-leukemic cells, but it was distributed throughout the cytosol in leukemic cells, this phenomenon might indicate the membranal structures of multivesicular body in the leukemic cells were unstable. So we presumed that instability of membrane structures in leukemic cells might participate in leukemogenesis. CQ could induce leukemic cells to apoptosis when Pnas-2's subcellular location was recovered, as we mentioned, some experts had reported that CQ could stabilize membranal structures, so this phenomenon might indicate the mechanism of chloroquine diphosphate treatment for leukemia: stabilizing membrane of multivesicular body, then inducing leukemic cells to apoptosis.

1116
MEMBRANE MOLECULE PROFILE AND MUTATIONAL STATUS IN ACUTE MYELOID LEUKEMIA

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Background. Flowcytomteric characterization of membrane molecule expression is important for the characterization of the leukemic cells in patients with acute myeloid leukemia (AML). The membrane molecule profile is useful mainly as a diagnostic tool, e.g. to distinguish between myeloblasts and lymphoblasts and to detect lineage-associated differentiation markers, whereas mutational analyses (especially FLT3 and NPM1 mutations) are important in the prognostic evaluation of AML patients. 

Aims. We wanted to use a limited number of well-characterized differentiation markers to characterize the membrane molecule profile of primary human AML cells derived from an unselected cohort of AML patients: to use bioinformatic tolls to subclassify the patients based on this profile, and finally to investigate whether there was an association between these patient clusters and biological or clinical patient characteristics. 

Methods. The membrane molecule profile was investigated by flow cytometry for 162 consecutive AML patients. Unsupervised hierarchical clustering methods were used to identify distinct immunophenotypic clusters. Results. The markers CD11c, CD13, CD14, CD15, CD33, CD34, CD45 and HLA-DR were used in an unsupervised hierarchical cluster analyse (Persons correlation, with complete linkage), and six major different patient clusters (I-VI) were then identified based on the expression levels. The figure describes the major characteristics for each cluster. The left panel describe the immunophenotypic characteristics. Black symbols indicate that the patients in that cluster mainly stained positive for the respective membrane molecule. Likewise white squares represent mainly negative expression of the respective marker.

Figure 1. Characteristics of the immunophenotypic clusters.

The French-American-British (FAB) classification was used for a morphological evaluation of leukemic cell differentiation. Grey squares represent either (i) FAB subclasses M0/M1/M2, or (ii) FLT3/NPM1 wild type being present in at least 80% of the patients in the cluster. In contrast, black squares represent monocyctic differentiation (FAB-M4/M5) or mutated FLT3/NPM1 alleles in at least 80% of patients in a specific cluster. White squares indicate no predominant morphology or mutational status in the respective cluster. The six clusters did not differ with regard to...
to age of the patients, hemoglobin levels or platelet count at diagnosis; in contrast, we observed a correlation as well as frequency of genetic abnormalities which differed between the clusters. Summary/Conclusions. Our results suggest that there is an association between mutational status and morphological as well as molecular signs of differentiation in primary human AML cells. Our bioinformatical analysis suggests that the membrane molecule profile can be used to identify different subsets among patients with similar genetic abnormalities. Only future studies can clarify whether the patient clusters differ with regard to chemosensitivity and prognosis, and the potential role of specific mutation in the for the phenotypic characteristics.

**1117**

**MONITORING OF MRD BY NPM1 MUTATIONS FROM MRNA AND GENOMIC DNA AND ITS COMPARISON WITH EXPRESSION OF WT1 GENE IN AML PATIENTS**

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Mutations of nucleophosmin 1 (NPM1) gene are the most frequent mutations in acute myeloid leukemia (AML) patients. In our Institute 20% AML patients have a mutation in NPM1 gene. Mutations in NPM1 gene were suggested as useful molecular markers for monitoring minimal residual disease (MRD) in AML patients. We compared monitoring of MRD by NPM1 mutations from both mRNA and genomic DNA with those of Wt-1 gene expression. The mutations of NPM1 gene and Wt-1 expression were estimated by a quantitative (RT-PCR), MRD was estimated in 28 AML patients with mutation A of NPM1 gene and with a high Wt-1 expression at diagnosis. The results showed a very good correlation between NPM1 mutations and Wt-1 expression and a clinical course of the disease. Comparison of results of MRD monitoring by NPM1 mutations from mRNA and genomic DNA showed in 10 AML patients a utility of both approaches. The results obtained from genomic DNA seem to fit better clinical data. Molecular relapse estimated by NPM1 mutations and Wt-1 expression preceded hematological relapse by 36 (median) days. The advantage of the NPM1 gene as the marker of MRD is his negativity in control healthy persons and in AML patients in permanent remission. The presented results showed a utility of both NPM1 mutations and Wt-1 expression as molecular markers of MRD in AML patients.

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**1118**

**ACQUIRED COPY NUMBER ALTERATIONS AND COPY NEUTRAL LOH IN ADULT AMLs WITH NORMAL KARYOTYPE IDENTIFIED USING SINGLE-NUCLEOTIDE POLYMORPHISM MICROARRAY**

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Background. FLT3, ITD, and NPM1 are recently discovered biomarkers dissecting prognostic subgroups of AML with normal karyotype (AML-NK). Genome-wide single-nucleotide polymorphism (SNP) analysis has devoted to reveal the previously unrecognized microdeletions and copy neutral LOH (CN-LOH). Genome-wide single-nucleotide polymorphism (SNP) analysis has devoted to reveal the previously unrecognized microdeletions and copy neutral LOH (CN-LOH).

Results. Total 26 adult AML-NK cases were subjected. Exclusion criteria were recurrent gene rearrangements of PML/RARA, BCR/ABL1, RUNX1/T1, MLL, and CBFB/MYH11 or mutations in FLT3, ITD, and NPM1 genes. DNA was extracted from the bone marrow cells collected at the time of diagnosis. Parallel study was available with the bone marrow samples at complete remission state from 3 cases. Genome-wide SNP analysis was performed with HumanCytoSNP-12 BeadChip (Illumina Inc.,USA). Nineteen CN-LOH regions detected by SNP array analysis were confirmed by qPCR. CN-LOH regions observed in 7 cases were smaller than 6.0 Mb. While, the other 8 regions observed in 8 cases (30.8%) were distinctively large, including 3p12.2-q11.22 (17.3Mb), 4p22.1-q52.2 (102.2 Mb), 7q11.23-q36.3 (352.2Mb), 8p24.3-p13.3 (35.6Mb), 11p14.3-p11.12 (29.8 Mb), 11p15.5-p11.2 (45.7Mb), 19q12-q13.43 (28.6 Mb and 31.3 Mb). CN-LOH in 19q12-q13.43 was recurrently observed in two different cases. Summary/conclusions. SNP microarray revealed additional copy number alterations at 19.2%, which were not detected in conventional karyotyping. We found significantly large CN-LOHs in 30.8% of AML-NK patients. Prognostic genes harbored in the large CN-LOHs, especially recurrent LOH region at 19q12-q13.43, need to be identified in further studies.
**Introduction.** Apoptosis is a controlled cell death mechanism of eradicating irreversibly damaged or unwanted cells from the body. Activation of this process is achieved by the shortening of telomeres at chromosomal endpoints to a critical length. Apoptosis is then induced by either caspase-dependent or-independent pathways. Highly activated telomerase, a reverse transcriptase may prevent shortening of telomeres to the critical length needed to activate apoptosis. This may result in uncontrollable proliferation of cancerous cells and lead to disorders like acute myeloid leukaemia. Curcumin, commonly known as tumeric, has been reported to have anti-tumour and apoptotic potential. It may influence telomerase activity and cause apoptosis via a caspase dependent pathway. **Aim.** To determine the effect of Curcumin on telomere lengths and caspase 3 and -4 activity in U937 acute myeloid leukaemia cells. **Methods.** The U937 cell line was cultured and treated with different Curcumin concentrations for 24- and 48 hours respectively. The relative telomere lengths were determined using Flow FISH. This assay was performed to determine how Curcumin influences telomerase. ELISA’s on caspase 3 and -4 activity in U937 acute myeloid leukaemia cells. **Results.** An increase in telomere lengths were observed with increasing Curcumin concentrations at 24 and 48 hour treatment periods. At a concentration of 10µM for the 24 hour treatment period, the telomeres were shortened. Caspase 3 and caspase 4 activity mostly increased with increasing Curcumin concentrations. Curcumin might influence telomerase and cause telomere shortening, however an alternative way of telomere elongation have to be considered in that case. Curcumin does influence the endoplasmic reticulum pathway of apoptosis as illustrated by increasing caspase 3 and caspase 4 activity and decreasing cell viability with increasing Curcumin concentrations.

**1121**

**EXPRESSION OF THE STEM CELL MARKERS CD133 AND CD90 ON BLASTS IN ACUTE MYELOID LEUKAEMIA AND IN MYELODYSPLASIA SYNDROMES IS ASSOCIATED WITH POOR PROGNOSIS**

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**Background.** CD133 and CD90 are surface markers expressed on various types of normal and cancer stem cells. Thus, we hypothesised that the expression of these two molecules could be of prognostic value in acute myeloid leukaemia (AML) and in myelodysplastic syndrome (MDS), as describe in some other cancers. **Aims.** In this study, we analysed the expression of CD133 and CD90 in AML and MDS blasts and in normal cases. The aim was to evaluate the stemness of the blasts and to correlate the expression of CD133 and CD90 to clinical outcome and to classical prognostic factors. **Methods.** The expression of CD133, CD90, CD34, CD38 and CD117 was evaluated by 8-colors flow cytometry on bone marrow samples collected at diagnosis for more than one hundred AML and MDS cases. CD34 blasts population was assessed by the level of expression and the different co-expression profiles of these markers. Clinical and biological data were also collected. **Results.** Blasts expressed CD133 in approximately 40% of cases, generally at a high level. CD90 was detected in 10 to 40% of cases, depending on the cut-off chosen (0 to 20% of cells stained) with a level of expression fainter than CD133. Significant correlations exist between the stem cell markers assessed. As expected, CD133+ blasts are more immature than CD133-, and express CD34 higher. The expression of CD90 is correlated to that of CD34 too. Interestingly, in several cases, we could isolate two distinct subsets in blasts: CD34+ and CD34- displayed different patterns regarding the expression of CD133 and CD90. In most cases CD133 was present on CD34+ blasts while CD90+ was preferentially expressed on CD34-. Co-expression of CD133 and CD90 in the same subset is very rare. We assessed the correlation between the expression of CD133 or CD90 and usual prognostic factors, and clinical outcome. The expression of CD133, as that of CD90, was significantly different between patients with a favourable, intermediate or unfavourable risk according to the FAB classification. When CD133 correlated also with the molecular prognostic profile. Moreover, CD133 and CD90 were associated with a higher risk of relapse. **Summary/conclusions.** CD133 and CD90 are expressed on normal haematopoietic stem cell (HSC) and other stem cells. They are markers of poor prognosis in AML and MDS cases but few studies analysed their expression in hematologic diseases. In this study we demonstrate that their expression correlates with prognosis factors as strong as cytogenetics. Interestingly, we observed different co-expression patterns for CD133, CD90 and CD34 within blasts. That heterogeneous population contains several subsets with different degree of stemness, as described in its normal counterpart. This could explain the relationship between CD133 and CD90 expression and clinical outcome. The prognostic value of the expression of CD133 and CD90 on blasts in AML and MDS must be confirmed and refined. Nevertheless, these encouraging results provide new insights into the leukemic stem cell characteristics. Moreover, as CD133 and CD90 correlate with other risk factors, they may represent novel prognostic markers in AML and MDS, particularly for patients with intermediate risk karyotype.

**1122**

**INVESTIGATING THE IMPORTANCE OF MSI2 IN MLL-FUSION INDUCED ACUTE MYELOID LEUKAEMIA**

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Chromosome translocations that disrupted the mixed lineage leukaemia (MLL) gene are associated with a unique subset of Acute Myelogenous and Lymphoblastic Leukaemias. MLL translocations are most prevalent in infant leukaemia, where they comprise 80% of cases of acute lymphoblastic leukaemia and 90% of cases in acute myeloid leukaemia. Expression of MLL-fusion proteins is known to induce malignant transformation of normal haemopoietic progenitor cells. To identify transcriptional target genes required for the immortalisation, previous work in the lab involved generating constitutively and conditionally immortalised primary mouse haemopoietic progenitor cells. Global gene expression analysis, upon loss of MLL-fusion protein, identified a number of genes that are differentially expressed. One of these genes was Msi2, an RNA binding protein, which is found to be expressed in haemopoietic stem cells. MSI2 prevents cell differentiation by binding to the mRNA of the cell fate determinant protein, Numb, and repressing its translation. Recent reports identified MSI2 to be highly expressed in both Acute Myeloid Leukaemia and Chronic Myeloid Leukaemia. Using the conditionally MLL-ENL immortalised cell lines, the expression of Msi2 and Numb was analysed by qPCR. Loss of MLL-ENL fusion protein resulted in significant reduction in Msi2 expression and an increased level of Numb mRNA. shRNA mediated knockdown of Msi2, in the constitutively immortalised MLL-ENL cell lines, led to decreased cell proliferation in culture and reduced colony formation in methylcellulose. Interestingly Numb over-expression, in these immortalised cells, had no effect on proliferation but reduced their colony forming capability. In vivo studies, examining the importance of Msi2 and Numb in MLL-fusion induced leukaemia, are currently under investigation.

**1123**

**REGULATION OF THE MTG16 LEUKAEMIA ASSOCIATED NUCLEAR CO-REPRESSOR GENE**

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The MTG16 gene, a member of the highly conserved ETO homologue family of co-repressors also containing ETO and MTG1, is implicated in hematopoietic development, in controlling erythropoiesis/megakaryopoiesis and is the 3'-partner of t(16;21) generating the leukemic AML1-MTG16 fusion gene. We examined MTG16 gene promoter regulation to shed light on hematopoietic functions. A TATA- and CCAAT-less promoter containing a GC box close to start site was identified. Mutation of an evolutionary conserved GATA -301 consensus binding site repressed promoter function. Furthermore, results from in vitro antibody-enhanced electrophoretic mobility shift assay and in vivo chromatin immunoprecipitation indicated specific binding of GATA-1 to the GATA -301 site. The leukaemia associated AML1-ETO fusion gene strongly suppressed all the ETO homologue promoters. In conclusion an evolutionary conserved GATA -301 site repressed promoter function. Furthermore, results from in vitro antibody-enhanced electrophoretic mobility shift assay and in vivo chromatin immunoprecipitation indicated specific binding of GATA-1 to the GATA -301 site. The leukaemia associated AML1-ETO fusion gene strongly suppressed all the ETO homologue promoters. In conclusion an evolutionary conserved GATA -301 consensus binding site repressed promoter function. Furthermore, results from in vitro antibody-enhanced electrophoretic mobility shift assay and in vivo chromatin immunoprecipitation indicated specific binding of GATA-1 to the GATA -301 site.
The presence of NPM1 mutations as an unfavorable factor for overall survival and for achieving complete remission in acute myeloid leukemia (AML).

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Background. Mutations in nucleophosmin (NPM1) are the most frequent abnormalities found in AML. Based on current findings, the presence of NPM1 mutations is associated with increased probability of complete remission (CR) and better overall survival (OS). Aims. To evaluate the incidence and the prognostic relevance of NPM1 mutations, their association with FLT3 mutations and other clinical characteristics.

Methods. Bone marrow or peripheral blood samples from 92 adult de novo AML patients (median age 50 years, range 16-75; M/F: 53/39) were studied. NPM1 mutations were detected by PCR method and the products were directly sequenced (Fahimi B, N Engl J Med 2005). FLT3 mutations were detected as previously described (Kiyoi H, Leukemia 1997; Yamamoto Y, Blood 2001). Results. NPM1 mutations were detected in 55/92 patients (18.5%). Three types of mutation were detected; type A in 15/17 patients (88.2%) while the remaining two patients were carriers of type D and type K mutations, respectively. NPM1 mutations were closely associated with normal karyotype in 85.7% of cases, and with CD34+ status (p=0.005). In 9/17 NPM1+ patients (53%), NPM1 mutations were associated with the presence of FLT3 gene mutations; in 6 (66.7%) NPM1+/FLT3+ patients, and in 3 (53.3%) patients with FLT3/D835 mutations. Complete remission was achieved in 7/17 NPM1+ patients. CR rate in NPM1+ patients was significantly lower than in NPM1-/FLT3- patients (p=0.02). When double positive NPM1+/FLT3+ patients (9 patients) and single positive FLT3+ patients were excluded from the calculation, CR rate was even lower (p=0.008) and CR was achieved in only 2/8 NPM1+ patients. No significant difference between NPM1+ and NPM1- patients was found concerning median duration of disease-free-survival (DFS) (10.5 vs. 12 months, p=0.38 Log-rank). Surprisingly, median OS among NPM1+ patients was significantly lower compared to NPM1- patients (4 vs. 10 months, p=0.015 Log-rank). Conclusions. The frequency of NPM1 mutations (18.5%) found in our study was slightly lower than in other previously published reports. The explanation for this may lie in the fact that 30% of our patients were younger than 40 and only 13.5% were older than 60 years, while NPM1 mutations have been shown to be more frequent in older patients. Young age structure of our cohort may also be the reason for unexpected finding that NPM1 mutations had an unfavorable impact on the CR rate and OS. It was previously shown that NPM1 mutations had favorable prognostic impact in older patients, especially those age ≥70 years, while in some other studies carried out on cohorts with different age structure, NPM1 mutations were associated with a higher relapse rate and poorer DFS. Association of NPM1 mutations with FLT3/ITD mutations, which have dominant adverse prognostic effect (also found in our study), may explain this unfavorable impact of NPM1 mutations. Recent data show that even in the presence of prognostically favorable NPM1 mutations, some AML patients may have an adverse outcome, suggesting that some other secondary genetic lesions may cooperate with NPM1 mutations influencing prognosis.

ACUTE MYELOID LEUKEMIA IN UNSELECTED COHORT OF ELDERLY PATIENTS: RESULTS OF INDUCTION VERSUS NONCURATIVE TREATMENT

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Introduction. The aim of our study was to analyze the results of treatment in elderly group of patients with AML diagnosed at the Institute of Hematology in Belgrade over a period of 6 years. We wanted to identify prognostic factors important for making decision how to treat elderly patients, with induction chemotherapy or with supportive or palliative therapy only. Material and methods. A retrospective analysis of 210 consecutive patients aged ≥65 years (median age 69, range 65-88) with acute myeloid leukemia (AML) diagnosed at a single center over the period of 6 years from January 2001 to December 2006 was performed. All patients who were included in this study provided informed consent with t(15;17), detected by FISH analysis, and 10 age and sex matched healthy controls were included in the study. Differences in patients and controls were analyzed for CEBPα expression by quantitative RT-real time PCR using TaqMan technology. Results. Thirty six (90%) patients out of the 40 showed low expression levels of CEBPα below the cutoff value with median of 0.51 (range:0.0007-0.96). Following therapy, full maturation of myeloid series was seen in 81 patients (77.5%). The 18 patients had higher median level of CEBPα of 0.6 (range: 0.22-26.7) compared to other 9 patients (22.5%) who showed partial response to therapy (median level: 0.03; range: 0.0007-0.15; p<0.0001). Conclusions. Our findings are highly suggestive that C/EBPα may have a role in the pathogenesis and prognosis of APL.
The serum level of LDH had no influence on OS. The OS significantly differed between patients with intermediate-risk and unfavorable cytogenetics (p<0.001) and between patients with and without trilineage dysplasia (p<0.01). The comorbidity score influenced OS, the patients with score ≥2 surviving significantly longer than those with score <2 (p<0.01). Conclusions. The subgroups of patients with AML show distinctive clinical characteristics suggesting a different biology of disease. A higher prevalence of comorbidity and a sharp diversity in biological features account for the poor response to therapy in this cohort of AML patients.
formed to AML after median 10.4 cycles of AZA (2-13). Conclusions. 1.- 90% of patients achieved a hematologic response. 2.- Time to response is early (3 months), although some patients response later (5 cycles or more). 3.- Efficacy and safety of AZA treatment is a valid alternative in low-int-1 risk MDS patients, although more studies are necessary. 4.- In secondary AML patients, AZA is an excellent alternative therapy in patients with co-morbidity and poor performance status.

1132
ASSOCIATION OF CD34 CELL SURFACE ANTIGEN EXPRESSION WITH CYTOMORPHOLOGICAL CHARACTERISTICS OF ACUTE PROMYELOCYTIC LEUKEMIA-BLASTS AND CLINICAL CHARACTERISTICS OF PATIENTS: ONE CENTER EXPERIENCE
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Background. Acute promyelocytic leukemia (APL) is characterized by leukemic cells blocked at the promyelocytic stage of granulocytic differentiation (APL-blasts). Two main cytological subtypes are recognized: classical hypergranular promyelocytic leukemia (M3) and the microgranular promyelocytic leukemia variant (M3v). A 5-parameter classification system (nucleus, granularity and Auer rods) leads to the distinction of 12 categories of APL-blasts with 3 additional categories (with basophilic granules, Chediak granules and Pelger-like maturing cells). Low or negative CD34 expression in addition to absent HLA-DR used to be the paradigm of the APL immunophenotype. However, higher CD34 expression can occur in APL and appears to be associated with leukocytosis, hypogranular morphology and poorer clinical outcome. AIM: to investigate association between cytomorphology and immunophenotypic expression of CD34 cell surface antigen of APL-blasts and their relationship with clinical and laboratory characteristics of patients with acute promyelocytic leukemia. Patients and Methods. Sixteen consecutive patients diagnosed with APL at Department of Hematology, University Hospital Merkur, between August 1998 and December 2010, were included in this study. Patients’ clinical and laboratory features, cytomorphological characteristics of APL-blasts and their immunophenotype were determined by flow cytometry and were then compared regarding to clinical and laboratory characteristics. Expression in more than 20% blast cells was required to define antigen positivity. RESULTS: Mean age of patients at diagnosis was 43.9 years (range: 18-78, SD14.9), 69% of patients were male. All evaluable patients had high CD13 and CD34 expression, with low HLA-DR expression, except one who had higher HLA-DR expression (HLA-DR(+) blasts: 27.6%). There was no statistically relevant difference between patients grouped according to CD34 expression according to gender, age or WBC counts. Mean value of hypogranular/agaranular APL-blasts in CD34(+) group was 34% (range: 9-60, SD 24.4), markedly higher than in the CD34(-) group, 11.5% (range 0-38, SD 13.7), with borderline statistical significance (Mann Whitney, p=0.055) (fig. 1). CD34(-) patients had significantly better overall survival (OS) than CD34(+) ones (p=0.02). The results of other published studies and point to the fact that higher CD34 expression and lower cytoplasmic granularity of APL-blasts are factors that seem to define a specific subgroup of patients with APL. Together with other diagnostic tools they could be of value in planning treatment of patients with acute promyelocytic leukemia.

Figure 1. Hypogranular APL-blasts and CD34 expression.
treated according to the EORTC (LAM 13 and LAM 17) protocols for pts above 60 yo. AMLs were classified according to the old FAB classification. Cytogenetic data were obtained by routine karyotype and additional FISH analysis since 1995. Complete remission was defined by morphology and flow cytometry on bone marrow smear. Karyotypes were stratified according to good and intermediate 1 versus intermediate 2 and poor prognosis (ELN recommendation). Statistical analyses using age and Karyotype were performed for the following outcomes: complete remission (CR), overall survival, median survival and disease-free survival. Results. 82 files were evaluable for cytogenetic data and outcome. Median age was 70 (60-86) yo. Because we are a referral tumour centre, all the pts had a PS <3 and no geriatric syndrome (falls, dementia, incontinence). The median follow up time was 40 (1-180) months. Overall survival for the whole population was 17% at 180 months with a median survival of 7 months. Median survivals of pts below 70 yo was significantly better (p < 0.0001) than pts above 70 yo (38 vs 7 months). Whatever the age, median survival was significantly better for pts in CR after the induction (48 vs 6 months) and presenting with a favourable Karyotype (5 vs 9 months). Taking into account the cytogenetic data and age, the median survival of AML pts below 70 yo with a favourable Karyotype was 64 months with 40% of CCR, very similar to the younger population. Conclusions. In our series of selected fit elderly AML pts, we confirm that cytogenetic data have a major impact of OS and CCR in pts between 60 and 70 yo. For patients above 70 yo, median survival remains unsatisfactory and these pts should be offered new alternative treatment approaches after an extended geriatric assessment.

CLOFARABINE IN THE TREATMENT OF POOR PROGNOSIS ACUTE MYELOID LEUKEMIA PATIENTS. A SINGLE INSTITUTION REVIEW

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Introduction. The incidence of Acute Myeloid Leukemia (AML) increases with age, and Cytarabine plus Anthracycline based treatments usually unfit for older patients, therefore worsening their prognosis and increasing mortality rate. Clofarabine is a purine nucleoside antimetabolite indicated in refractory pediatric Lymphoblastic Leukemia, but with demonstrated efficacy in AML patients in combination with Cytarabine, with tolerable side effects, becoming an option for those patients with relapse/refractory AML, as well as for those non candidates for aggressive approaches. Aims. To evaluate the effectiveness and toxicity profile of Clofarabine in AML elderly patients. Methods. This is a single-center retrospective review of patients with AML treated with Clofarabine based regimens in patients aged 60 and above. Main points were complete response as IWRR2003 criteria and toxicity as CTCAE v3.0 del NCI criteria. Results. In the period between January 2007 and December 2010, 6 AML patients above the age of 60 began treatment with Clofarabine (30 or 20 mg/m2/day x 5 days) plus AraC (500 mg/m2/12h x 8 doses). The mean age was 68.5 (63-77), and the ratio male/female 4/2. AML profile was 6 cases. Salvage therapy: 4 patients. Five of the 6 patients (83%) presented poor prognosis cytogenetic alterations. Effectiveness. Two patients (33%) obtained complete remission, with a progression free survival of 7 and 4 months (mean 5.5), and an overall survival of 9 and 10 months (mean 9.5), respectively. No effective data of 30 mg/m2/day dose patients are collected, because they were exits within the first cycle. Toxicity. Hematologic toxicity was observed in all patients, grade 4 Neutropenia, grade 4 thrombocytopenia and anemia transfusion-dependent.

The 2 patients treated with 30 mg/m2/day x 5 days were exits because of septic shock during the induction cycle. There was no correlation between number of previous lines received and toxicity. No relevant extrahematologic toxicity was reported in patients treated with 20 mg/m2/day x 5 days. Conclusions. Our experience confirms that Clofarabine at 20mg/m2/day (instead of the initially proposed 40 mg/m2, or the 30 mg/m2 we have tried before) x 5 days plus AraC 500 mg/m2/12 x 8 doses, is effective, obtaining a complete remission rate of 33% in these poor prognosis settings, and well tolerated. These preliminary results encourage the testing of Clofarabine in AML patients, and reveal the need of post remission consolidation or maintenance to increase PFS and overall survival.

HIGH-DOSE DAUNORUBICIN VERSUS STANDARD-DOSE IDARUBICIN REMISSION-INDUCTION THERAPY FOR NEWLY DiAGNOSED PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Continuous infusion of cytarabine with an anthracycline has been the mainstay of therapy in acute myeloid leukaemia (AML). Recent studies suggested that intensification of anthracycline dose might improve complete remission rates in AML patients. Results of high-dose daunorubin induction induction studies demonstrated improvements not only in complete remission rates but also overall survival. In this retrospective study we investigated the efficacy of remission-induction therapy for adult patients with newly diagnosed AML either as high-dose daunorubicin (90 mg/m2 daily for 3 days) or idarubicin (12 mg/m2 daily for 3 days) in combination with 100 mg/m2 cytarabine by continuous infusion daily for 7 days. Patients achieving complete remission received intensive postremission therapy that consisted of 4 courses of high-dose cytarabine. Nine of the 20 patients had high-dose daunorubicin. Remaining 11 patients received standard-dose idarubicin as a part of induction therapy. The median age of all patients was 40.5 years (range, 19-67). Cyto- genetic risk groups were similar in both groups. There were no significant differences in complete remission rate between two induction groups (P = 0.2). The median (± SEM) follow-up time was shorter in daunorubicin induction group (7 ± 1.1 months) compared to idarubicin induction group (13 ± 5.2 months) (P=0.04). Relapse rates were not different between two groups (P=0.4). The incidence and the intensity of adverse effects were comparable. Our data revealed that high-dose daunorubicin and standard-dose idarubicin therapies were equally effective as remission-induction treatment however longer follow-up with larger series is definitely needed.

ADAPTIVE DESIGN OF VALOR, A PHASE 3 TRIAL OF VOSAROXIN OR VOREXOVIN IN COMBINATION WITH CYTARABINE FOR PATIENTS WITH FIRST RELAPSED OR REFRACTORY ACUTE MYELOID LEUKEMIA

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Background. Patients with relapsed or refractory AML have a short median overall survival (OS) of 3 to 6 months. Vosaroxin (formerly vorexoxin), a first-in-class anticancer quinolone derivative, showed promising activity in combination with cytarabine in a single-arm phase 2 trial in this patient population (N=69). Median OS was 7.1 months, combined complete remission (CR) rate (CR+CRp+CRi) was 29%, CR rate was 25%, median leukemia-free survival 4.15 months (range 14.5-07), and 30-day all-cause mortality was 3%. Aims: Given the uncertainty of basing study design on historical data, and the inherent challenges of extrapolating from a phase 2 dataset to a larger, multinational study, an adaptive design was incorporated into the VALOR phase 3 trial to mitigate the risk of negative outcome where statistical significance is not reached at the final analysis, yet where a truly clinically meaningful benefit could be detected if sample size adjustment were made at interim analysis. An adaptive study design allows staged commitment of patients and
The increase substantially reduces the risk of failing to detect a clinical difference in OS between treatment arms, requiring a larger initial commitment of patients. Methods. Adaptive Trial Design: VALOR is a phase 3, randomized, controlled, double-blind, multinational clinical study of the efficacy and safety of vosaroxin and cytarabine versus placebo and cytarabine in patients with first relapsed or refractory AML. Vosaroxin (90 mg/m2) or volume-equivalent of placebo is administered on days 1 and 4 in 25.6×109/L (range: 0.6-183) on 30 pts in induction (mean duration 15 days), and in 28 (13 days), 26 (10 days), and 13 (8 days) pts during consolidation, respectively. Hepatotoxicity grade 2-4 (with MTHFR) developed in 10 pts; severe cardiotoxicity in 2 pts; genital ulcer one pts; pneumonia 7 pts (5 Aspergillosis); thrombosis in 6 pts. Patients with thrombosis were older (52 vs. 41 years; p=0.04). Conclusions. PETHEMA LPA-99 proved effective in our APL patients concerning CR rate, OS, DFS, toxicity and relapse rate. High rate of early death could be attributed to the numerous high-risk pts and increased rate of DIC. WBC >10x109/L, DIC score ≥5 and CD15 negativity were poor prognostic factors for early death. Reduction of the incidence of early deaths is mandatory and requires a timely diagnosis and better management of both DS and bleeding.

CHEMOTHERAPY WITH LOW-DOSE MERCAPTOPURINE IN ELDERLY, UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background. In acute promyelocytic leukemia (APL) a cure rate of 75-80% can be anticipated with a combination of all-trans retinoic acid (ATRA) and anthracyclines. Aim. To evaluate the efficacy of PETHEMA LPA-99 protocol in elderly, unfit patients with AML. Methods. From 2004 to 2010, 42 consecutive APL patients (pts) confirmed either by t(15;17) or RARA/APR were treated with PETHEMA LPA-99. The median follow-up was 32 months (range: 1-78). Results/Patients characteristics. Median time from the first symptoms to diagnosis was 23 days (range: 5-90). Pretreatment pts characteristic were as follows: median age 42 years (range: 21-69), 22/42 male; mean WBC 25.6×109/L (range: 0.6-183); mean platelet count 34x109/L (range: 4-101); hypergranular form in 39/42 (95%) pts; PETHEMA risk stratification: high 17/42 (40%), intermediate 17/42 (40%), low 8/42 (20%); mean D-dimer 3606 μg/L (range: 996-11340). DIC was confirmed in 32/42 (85%) pts, mean score 6 (range: 5-7). Additional cytogenetics abnormalities were detected in 8/42 (19%) pts (trisomy 8 in 4/8). Therapy results: 33/42 (76%) pts achieved complete remission (CR). Induction death occurred in 9/42 (21%) patients due to: differentiation syndrome (DS) - 4/42 (9.5%), central nervous system hemorrhage- 4/42 (9.5%) and infection- 1/42 (2.3%). Early died pts had higher WBC (55.9x109/L vs. 14.1x109/L, p=0.014), higher percentage of peripheral blood (PB) promyelocytes (15.0% vs. 9.59% vs., p=0.05) and higher DIC score (6.32 vs. 5.85, p=0.014). All died patients were CD15 negative and with a DIC score of >5. DS occurred in 11 pts (26%) with a median onset time of 4 days (range: 1-24) and a median WBC of 25.9×109/L (range: 1.4-87.7x109/L). DS was severe in 9 cases/4 fatal and moderate in 2. Pts with DS had significantly higher percentage of PB promyelocytes (48% vs. 10% vs., p=0.04). Mean platelet survival rate was expressive for DS (p=0.048). Three relapses occurred after the third consolidation, during a maintenance and 6 months after the maintenance termination, respectively. Mean time to relapse was 14 months (range: 2-31). The 26/42 (62%) pts are alive in first continuous CR. Three-year overall survival (OS) is 76% and disease free survival (DFS) 22%. Tolerance and toxicity according NCI: Leuko-thrombocytopenia grade 3-4 was registered in 30 pts in induction (mean duration 15 days), and in 28 (13 days), 26 (10 days), and 13 (8 days) pts during consolidation, respectively. Hepatotoxicity grade 2-4 (with MTHFR) developed in 10 pts; severe cardiotoxicity in 2 pts; genital ulcer one pts; headache grade 3-4 2 pts; pneumonia 7 pts (5 Aspergillosis); thrombosis in 6 pts. Patients with thrombosis were older (52 vs. 41 years; p=0.04). Conclusions. PETHEMA LPA-99 proved effective in our APL patients concerning CR rate, OS, DFS, toxicity and relapse rate. High rate of early death could be attributed to the numerous high-risk pts and increased rate of DIC. WBC >10x109/L, DIC score ≥5 and CD15 negativity were poor prognostic factors for early death. Reduction of the incidence of early deaths is mandatory and requires a timely diagnosis and better management of both DS and bleeding.

CHEMOTHERAPY WITH LOW-DOSE MERCAPTOPURINE IN ELDERLY, UNFIT PATIENTS WITH ACUTE MYELOID LEUKEMIA (AML)

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Background. In acute promyelocytic leukemia (APL) a cure rate of 75-80% can be anticipated with a combination of all-trans retinoic acid (ATRA) and anthracyclines. Aim. To evaluate the efficacy of PETHEMA LPA-99 protocol in elderly, unfit patients with AML. Methods. From 2004 to 2010, 42 consecutive APL patients (pts) confirmed either by t(15;17) or RARA/APR were treated with PETHEMA LPA-99. The median follow-up was 32 months (range: 1-78). Results/Patients characteristics. Median time from the first symptoms to diagnosis was 23 days (range: 5-90). Pretreatment pts characteristic were as follows: median age 42 years (range: 21-69), 22/42 male; mean WBC 25.6×109/L (range: 0.6-183); mean platelet count 34x109/L (range: 4-101); hypergranular form in 39/42 (95%) pts; PETHEMA risk stratification: high 17/42 (40%), intermediate 17/42 (40%), low 8/42 (20%); mean D-dimer 3606 μg/L (range: 996-11340). DIC was confirmed in 32/42 (85%) pts, mean score 6 (range: 5-7). Additional cytogenetics abnormalities were detected in 8/42 (19%) pts (trisomy 8 in 4/8). Therapy results: 33/42 (76%) pts achieved complete remission (CR). Induction death occurred in 9/42 (21%) patients due to: differentiation syndrome (DS) - 4/42 (9.5%), central nervous system hemorrhage- 4/42 (9.5%) and infection- 1/42 (2.3%). Early died pts had higher WBC (55.9x109/L vs. 14.1x109/L, p=0.014), higher percentage of peripheral blood (PB) promyelocytes (15.0% vs. 9.59% vs., p=0.05) and higher DIC score (6.32 vs. 5.85, p=0.014). All died patients were CD15 negative and with a DIC score of >5. DS occurred in 11 pts (26%) with a median onset time of 4 days (range: 1-24) and a median WBC of 25.9×109/L (range: 1.4-87.7x109/L). DS was severe in 9 cases/4 fatal and moderate in 2. Pts with DS had significantly higher percentage of PB promyelocytes (48% vs. 10% vs., p=0.04). Mean platelet survival rate was expressive for DS (p=0.048). Three relapses occurred after the third consolidation, during a maintenance and 6 months after the maintenance termination, respectively. Mean time to relapse was 14 months (range: 2-31). The 26/42 (62%) pts are alive in first continuous CR. Three-year overall survival (OS) is 76% and disease free survival (DFS) 22%. Tolerance and toxicity according NCI: Leuko-thrombocytopenia grade 3-4 was registered in 30 pts in induction (mean duration 15 days), and in 28 (13 days), 26 (10 days), and 13 (8 days) pts during consolidation, respectively. Hepatotoxicity grade 2-4 (with MTHFR) developed in 10 pts; severe cardiotoxicity in 2 pts; genital ulcer one pts; headache grade 3-4 2 pts; pneumonia 7 pts (5 Aspergillosis); thrombosis in 6 pts. Patients with thrombosis were older (52 vs. 41 years; p=0.04). Conclusions. PETHEMA LPA-99 proved effective in our APL patients concerning CR rate, OS, DFS, toxicity and relapse rate. High rate of early death could be attributed to the numerous high-risk pts and increased rate of DIC. WBC >10x109/L, DIC score ≥5 and CD15 negativity were poor prognostic factors for early death. Reduction of the incidence of early deaths is mandatory and requires a timely diagnosis and better management of both DS and bleeding. 
ing the treatment period. All patients with cytogenetic abnormalities were poor responders. The patients who failed to obtain response had survivals of 1–5 months. In our opinion there might be a group of elderly patients with AML that would have a real benefit in terms of survival and quality of life using low-dose 6 mp therapy. This hypothesis has to be confirmed by further studies on larger patient population combined with cytogenetic and molecular analysis.

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EVALUATION OF TREATMENT OF ACUTE PROMYELOCYTIC LEUKEMIA (APL) BY A PROTOCOL INCLUDING ALL-TRANS RETINOIC ACID (ATRA)
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Introduction. The APL is a rare form of acute myeloid leukemia (AML) but potentially serious because of the risk of bleeding involving immediate threat to life. The introduction of all-trans retinoic acid (ATRA: Tretinoin), whose first results were published in 1997, has completely revolutionized the treatment and prognosis of this disease Material and methods. From January 2000 to December 2009, 39 patients (pts) with APL were diagnosed from a total of 508 AML (16%). The diagnosis was made on the morphological study of bone and blood smear according to FAB classification criteria. Including ATRA treatment was applied in 37 pts. Among the remaining 13 pts, 8 died before any treatment of hemorrhagic syndrome and 5 had received only chemotherapy (CT). The median age of 37 pts was 30 years (12-66), 26 were female and 11 male (sex ratio: 0.5). At diagnosis, all pts but one, have an hemorrhagic syndrome, 22 pts/37 (56%) were febrile, no tumor syndrome was observed. On haematological, thrombocytopenia is consistent with a median of 21.10^9/L (2.65), 16 pts (43%) have leucocytosis with yield count (WBC) more than 10.10^9/L part of the high-risk prognostic group (Sanz, Blood 2000). Symptomatic treatment involving transfusions of platelets to maintain a platelet count greater than 30.10^9/L and fresh frozen plasma is associated with specific treatment protocol follows: ATRA 45 mg/m2/day only if rate WBC ≤ 5.10^9/L. The 3+ protocol is started at day 5 if WBC ≤ 6.10^9/L at day 10 if WBC ≥ 10.10^9/L or after complete remission (CR) and 2 courses of chemotherapy followed by maintenance therapy comprising ATRA. Among the 37 pts, eight received ATRA alone for induction and 29 of ATRA + CT; all pts received consolidation therapy and maintenance therapy. The median follow-up was 60 months (15-112). Results. CR was achieved in 30 pts/37 (81%), 6 pts (16%) died during induction within 5-19 days (25 septic shock, an acute respiratory distress, 1 hemorrhage cerebrospinal meningi, 2 of unknown cause), 1 pt died in failure at 3 months. During follow-up among the 30 CR, 5 pts died (3 due to septic shock after a course of consolidation, after an allograft and 1 after early relapse at 5 months). In total, 25 pts/37 (67%) are in persistent CR. The actuarial overall survival (OS) of 37 remis is 65% at 5 years (65%) and the other 4 pts the survival varied between 2-7 month. In our opinion it is not possible to make any meaningful comparisons during chemotherapy (severe infections - 6 cases, hemorrhage - 2 cases, severe anemia associated with heart failure or cardiac ischemia - 3 cases). After failure of conventional therapy the therapy was changed to low-dose 6MP oral treatment (100 - 150 mg/week) in association with valproic acid (Convules 900 mg/day, orally). Treatment decision was made after consulting the patients and their families. Results: Two patients who had complete remissions with duration of 6 and 36 month, respectively (both AML secondary to MDS, 1 with AML6 subtype and t(12;15)). The other 6 patients did not achieve hematologic responses, but 2 of them had stable disease with survival of 14 and 20 month despite the lack of remission. For the other 4 patients the survival varied between 3 - 7 month. In our opinion that some elderly with AML could respond to the association of valproic acid and mercaptopurine and even in the absence of remission there might be some benefit in terms of survival. This hypothesis has to be confirmed by further studies on larger patient population combined with cytogenetic and molecular analysis.
CLOFARABINE-BASED REGIMENS AS SALVAGE CHEMOTHERAPY FOR ADULT ACUTE MYELOBLASTIC LEUKEMIA - EXPERIENCE OF ROMANIAN WORKING GROUP FOR ADULT ACUTE LEUKEMIA STUDY (RWGALS)

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Background. Clofarabine (2-chloro-20-fluoro-deoxy-9-b-D-arabinofuranosyladenosine) is a second-generation nucleoside analog, which was developed as a hybrid molecule to combine the most favorable pharmacokinetic properties of both fludarabine and cladribine. Aims: The encouraging activity in the heavily pretreated study group, tolerable toxicity profile, and associated pharmacokinetic and pharmacodynamic parameters led us to investigate clofarabine in relapsed or resistant acute myeloblastic leukemia (AML). Materials and methods: We had studied 9 patients with relapsed AML (7 patients) or resistant AML (2 patients) diagnosed in 2010. Three patients had relapsed secondary AML post SMD, other 5 had relapsed high risk AML. One patient had relapsed AML after allotransplant, matched related donor (twin brother). There were 3 female and 6 male, median age 53 years. Clofarabine based regimens was variable: 4 patients received idarubicin 12 mg/sm/3 days combined with Clofarabine 52 mg/sm/5 days, 3 patients received monotherapy with clofarabine, 52 mg/sm/d 5 days, 1 patients received Clofarabine 52 mg/sm/d 5 days combined with low dose AraC (7 days) and 1 patient received Clofarabine 32 mg/sm/d 4 days combined with AraC 1g/sm/d 4 days. Results: Clofarabine, given at 52 mg/sm daily for 5 days per course combined with idarubicin had a significant antileukemic activity in heavily previously treated relapsed AML, with CR an average duration of 23 days. Nonhematological side effects were tolerable and mostly reversible. Hematological (thrombocytopenia and neutropenia) grade III-IV toxicity had 89% patients, with an average duration of 28 days. Summary: We found that patients younger than 40 years were much better tolerated treatment with clofarabine than older people. Also, treatment toxicity in patients with early relapse was shorter in duration than those with full relapse. These observations could lead to reconsider the indication of treatment with clofarabine in adult AML.

RESULTS

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DE NOVO ACUTE MYELOID LEUKEMIA WITH TRILINEAGE DYSPLASIA. HEMATOLOGICAL AND BILOGICAL FEATURES. SINGLE CENTER EXPERIENCE

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Background. Current WHO classification of AML describes the entity of AML with trilineage dysplasia. This type of leukemia is hard to diagnose since many patients lack enough morphological details due to expansion of leukemia. Thus, not many biological features were described in selected group of patients. Aim. The aim of our study was to analyze patients diagnosed with AML with dysplasia according to WHO (2008/2009) classification and to compare their features with similar patients from the same period within single academic institution. Patients. Between 1998-2003, we have identified 50 pts with de novo AMLtnd among 270 leukemia cases diagnosed. Comparison was made with 80 patients without dysplasia (AMLt) treated in the same period, with the chemotherapy based on MRC AML 10 trial. We analyzed hematological, morphological, cytogenetic and biological data such as proliferation and spontaneous and drug-induced apoptosis (in vivo apoptosis after 48h of therapy), bcl-2 positivity by immunohistochemistry. Also we analyzed response to treatment.

RESULTS

Results. The incidence of this type of AML is roughly 18% of all AML. Patients with AMLtd are elder than others (50.3 vs 42.2). They had in general more myeloid leukemia (M2) than monocytic forms. The incidence of aleukemic type is much more common in AMLtnd (54 vs 16%), meaning that WBC and blast counts are also lower. In morphology, when enough myeloid tissue besides leukemia was present, all morphological dysplastic changes in all lineages were found, with prominent dysplastic changes in erythroid and granulocytic series (megakaryoblastic E lineage, dysplastic nuclei; pseudopelgers, disturbed or hypogranulated G lineage). Karyotype revealed that AMLtnd had typical cytogenetic lesions affecting chromosomes 5, 7, 8, multiple aberrations (52%) but also normal karyotype, no prognostically favourable karyotype was found. Remission rate was lower 42.5% vs 73%, with higher induction death 22.5% and longer aplasia (19 days). Median OS was shorter (9 vs 15 mths) with 11 vs 28% at OSY. Patients with AMLtnd had higher number of proliferative cells (Ki67 7.5 vs 6.5%), and this group also had higher incidence of patients with Ki67>50% (43% vs 16%). There was no difference in spontaneous apoptotic rate 3.5% vs 3.5%, but hemotherapy induced apoptosis was higher in patients without dysplasia 5.8±5.2% vs 3.6±3% in AMLtnd. Bcl-2 positivity (IHC) revealed that 65% with AMLtnd were positive compared to 45% in AML group (p<0.07).

Conclusions. Patients with AML with trilineage dysplasia had characteristic form of disease, with lot of poor prognosis features, and low response to treatment. In case that they are eligible, they probably will benefit from more intensive treatment approaches.

GENE MUTATION IN PAIRED INITIAL PRESENTATION AND RELAPSE SAMPLES FROM ACUTE MYELOID LEUKEMIA

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Background and Aims. Several gene mutations were found in acute myeloid leukemia (AML) and were expected to be used as prognostic factors and minimal residual disease (MRD) markers. We have studied FLT3, NPM1, CEBPA, IDH1/2 gene mutations in paired samples at initial presentation and relapse of AML to ascertain the biological meanings of these mutations and to evaluate whether they can be used as MRD marker. Methods. We analyzed paired samples at initial presentation and relapse from 30 adult patients with de novo AML who diagnosed at Nippon Medical School from 2000 to 2010. Bone marrow or peripheral blood samples containing 20% or more blast cells obtained were used for mutation analyses. Mutation analyses were performed for FLT3ITD by PCR amplification, FLT3TKD by PCR-RFLP, and NPM1, CEBPA, IDH1/2, mutations by direct sequence. To validate sequencing results, PCR products were inserted into the pCR2.1-TOPO vector using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA). Recombinant plasmids isolated from 8 to 12 white colonies were sequenced. Results. FLT3ITD, FLT3TKD, NPM1, CEBPA, IDH1/2 mutations were detected in 9 (30.0%), 3 (10.0%), 3 (10.0%), 10 (33.3%), 1 (3.3%), 2 (6.7%) samples at initial presentation samples, and 8 (27.6%), 0 (0%), 7 (23.3%), 1 (3.3%), 2 (6.7%) samples at relapse, respectively. Among 24 available samples for chromosomal analysis at relapse, 16 (66.6%) showed additional chromosomal aberrations, 6 (25.0%) changed to be complex chromosomal aberrations. Chromosomal instability at relapse was observed in many cases, but frequency of gene mutations at relapse was lower than those at initial presentation. About 40% of AML patients at relapse did not have these gene mutations detected at initial presentation. CEBPA mutation
was found in one paired sample. IDH1/2 mutations were detected in 2 paired samples of patients. Interestingly, 3 of 9 patients with Flt3ITD mutation at initial presentation had no Flt3 ITD mutation at relapse. Flt3/TKD mutations were found in 3 patients at initial presentation, but all of them were lost in these three cases at relapse. Among the 10 patients with NPM1 mutation at initial presentation, 3 had no detectable mutations at relapse. These results indicate that Flt3ITD, Flt3TKD, and NPM1 mutations should be carefully used for the detection of MRD. Furthermore, among the 6 patients with NPM1 mutation without Flt3ITD mutation that is known as favorable factor, Flt3ITD mutations were detected in 2 patients at relapse. There was a possibility that detection sensitivity for Flt3ITD is not sufficient by PCR amplification method. Conclusions: Our study showed that about 65% of the patients with Flt3ITD mutation had the same mutation at relapse, which means that these mutations may contribute to expand resistant clones. On the other hand, detection of Flt3ITD mutation only at relapse suggested that a minor clone harboring Flt3ITD mutation might be overlooked at diagnosis. We have developed a highly sensitive mutation detection method (15th. Congress of European Hematology Association, 2009, Barcelona, Spain) and studies to clarify these findings are ongoing.

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GEMTUZUMAB-OZOGAMICIN AS POST-CONSOLIDATION THERAPY IN ELDERLY PATIENTS WITH ACUTE MYELOID LEUKEMIA: A PILOT STUDY
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Background. The optimal post-remission treatment for elderly patients with acute myeloid leukemia (AML) is currently unknown. Autologous transplantation is not always feasible in this setting because unsuccessful full mobilization and comorbidities. The high relapse risk of the disease raise the issue of exploring alternative consolidation regimens. Aims. We evaluated safety and efficacy of low dose Gemtuzumab-Ozogamicin (GO) as post-remission late consolidation therapy in a cohort of 19 consecutive patients, failing mobilization, enrolled in a pilot prospective study. Between June 1999 and February 2010, we observed 152 elderly patients, aged more than 60 years and affected by AML. One hundred and two patients (67%) were considered fit for aggressive therapeutic protocols with bone marrow allotransplantation while poor mobilizers received a second consolidation: 19 with GO, 20 with Autologous Transplant, and 6 with chemotherapy alone for patient decision. Five patients with a family donor received Allogeneic Transplant and were not included in this analysis. GO was well tolerated: no major adverse events were seen. We overall observed 18 WHO grade III/IV adverse events were all transitory and included hematological toxicity (n = 17), hypertransaminasemia (n = 1). Eight patients (42%) relapsed after GO consolidation and received a GO reinduction: five eventually died after a median follow-up of 13 months while three are still alive with a median follow up of 10 months. Five out of 20 patients died of transplant related toxicity (25%) and of the remaining 15 relapsed (60%) after autologous transplant. All nine patients died of progressive disease. Five of the 6 patients receiving chemotherapy (83%) relapsed and died. Five years Overall survival was 22% and Disease Free Survival 29.5% in the whole cohort of patients (median follow-up: 29 months). The landmark analysis showed a superior outcome in patients receiving GO with a 60% 5 yrs OS and DFS (median follow-up: 60 months) in comparison with patients receiving autologous transplant (25% 5 yrs OS and DFS with median follow-up 70 months) and chemotherapy (17% 5 yrs OS and DFS with a median follow-up of 77 months) (p = 0.009). Conclusions: Patients receiving GO had a better outcome in comparison with patients receiving autologous transplant and chemotherapy. High percentages of poor mobilizers and transplant related mortality seem to jeopardize autologous transplant feasibility and safety in elderly patients. GO showed to be an alternative and feasible choice in this setting.

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BLASTIC PLASMACYTOID DENDRITIC CELL NEOPLASIA (BPDCN): REPORT OF 2 CASES
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Background. BPDCN is a rare hematopoietic disease that typically involves the skin, lymph nodes, peripheral blood and bone marrow. Prognosis is dismal with a median overall survival of only 12-14 months despite aggressive chemotherapy. We report 2 cases of BPDCN associated with dysplasia. CASE 1: A 68-year-old man consulted for asymptomatic, infiltrated, disseminated skin lesions that had appeared during the previous 6 months, with progressive onset of anaemia (hemoglobin 112 g/L) and leukopenia (2.8 x10^9/L). Platelet count and lactate dehydrogenase were normal. A skin biopsy revealed a dermal infiltration of mononuclear medium-sized mononuclear cells with expression of CD4 and CD56, but not CD3 and CD20. Bone marrow aspirate was infiltrated by 48% medium-large sized blasts with a peculiar morphology. The nucleus was irregularly shaped and the chromatin was lacy with a blastic appearance. The cytoplasm was large, grey and agranular, with pseudopodium-shaped expansions and occasionally vacuoles. Myeloperoxidase was negative and esterase was not conclusive. Trilineage dysplasia was observed. Flow cytometry immunophenotyping confirmed the diagnosis of BPDCN (positivity for CD4, CD56, CD123, and HLA-DR, negativity for B, T and myelo-monocytic markers). Fluorescence in situ hybridization (FISH) revealed +12, del (13q14.3) and del (13q34). The karyotype was not available. FLAG-Ida (fludarabine, cytarabine, idarubicin and G-CSF) was administered as induction therapy. Consolidation therapy with FLAG-Ida is ongoing and the patient is scheduled for autologous hematopoietic stem cell transplantation. He is currently in remission, with negative minimal residual disease documented by flow cytometry and FISH although with persistence of trilineage dysplasia and cytopenias. CASE 2: A 78-year-old man was monitored for a medical history of ischemic heart disease and refractory anemia that progressed to refractory anemia with excess blasts type 1 (RAEB-1). His clinical course over the previous 4 years has been stable. He presented with a purple asymptomatic cutaneous lesion on his left shoulder and weakness. A blood count revealed neutrophils 0.4 x 10^9/L, platelets 16 x 10^9/L, and hemoglobin 76 g/L. Cytological examination of bone marrow showed 79% blast cells with the same morphology and cytochemistry as case 1, as well as myelodysplasia of the 3 cell lines. Immunophenotyping revealed BPDCN (positivity for CD4, CD56, CD123, HLA-DR, negativity for B, T and myelo-monocytic markers). A cytogenetic study revealed a complex karyotype. Cutaneous biopsy showed massive dermal infiltration by BPDCN. In view of the patient’s age and comorbid conditions, supportive care was provided and he died 4 months later. Conclusions. The clinical presentation of both patients was very similar, with cutaneous involvement and cytopenia. Morphologic and cytochemical features were characteristic and similar. Immunophenotyping was diagnostic. Both patients had chromosomal changes. Survival is usually poor without treatment. This justifies aggressive protocols with bone marrow allotransplantation.
ACUTE LEUKEMIA: HOW WELL DO HISTOLOGY AND FLOW CYTOMETRY CORRELATE AT DIAGNOSIS?

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Background. Acute leukemia is a common haematological malignancy, which comprises acute myeloid leukemias (AML), acute lymphoblastic leukemia (ALL) and acute leukemia of ambiguous lineage (AML-LAL). Diagnosis is by bone marrow trephine biopsy (BMTB), flow cytometry (FC), bone marrow aspirate, peripheral blood investigations and cytogenetics. Review of the available literature showed correlation between FC and histology of 88% when sampling lymph nodes in non-Hodgkin lymphomas (El Sayed et al. 2008) and 89% in marginal zone lymphoma when sampling bone marrow (Boveri et al. 2009). No literature could be found on this parameter in acute leukemias. Aim: To compare BMTB with FC in patients with suspected acute leukaemia at diagnosis. Methods: Diagnostic BMTB and FC reports, from May 2004 to December 2010, from patients suspected to have an acute leukaemia and where both investigations were performed.

Results. A total of 46 cases were studied. In 44 cases (96%) both FC and BMTB were reviewed. Results were included if the FC and BMTB samples were sent within two weeks of each other. FC panels included antibodies to CD10, CD117, CD13, CD34, CD33, myeloperoxidase, HLA-DR, CD45, TdT, CD79a, cCD22, CD2, cyCD3, CD7, CD14 and CD15. Immunohistochemical panels on BMTB included CD4, CD117, myeloperoxidase, HLA-DR, TdT, CD68, CD15, CD35, glycophorin A, CD61/CD42b, CD20, CD79a, PAX5 and CD8. Results. A total of 46 cases were studied. In 44 cases (96%) both FC and BMTB reported an acute leukaemia. In the remaining 2 cases - 1 was reported as AML on FC and as myelofibrosis with dysplastic megakaryocytes on BMTB, the other was reported as suspicious of AML on BMTB and as lymphocytosis on initial FC, though the subsequent FC in this pic had clear blast features. AML-LAL were correlated in 96% of cases of acute leukemia: Both FC and BMTB reported precur sor B-cell ALL in 5 cases and T- cell ALL in 1 case. In 35 cases, both FC and BMTB reported AML. Of these 4 were reported as acute promyelocytic leukemia; 2 by both FC and BMTB, 1 by FC only and 1 by BMTB only. In 1 case, the BMTB suggested megakaryoblastic differentiation which was not confirmed by FC, this sample was reported as being haemoaemoid. In 1 case, the BMTB suggested megakaryoblastic differentiation which was not confirmed on FC. In 2, FC reported poorly differentiated acute leukemia, and in 1 of these cases BMTB reported acute leukaemia - not otherwise specified (NOS), in the other case BMTB reported pro-B-cell ALL. In 1 FC reported mixed phenotype acute leukemia and the BMTB reported AML-NOS. Conclusions. BMTB and FC correlated in 96% of cases of acute leukemia (44/46). A concordance of 95% was seen in the diagnosis of the subtypes of acute leukemia (42/44). Within AML, where lineage/subtype-specific subtype differentiation was attempted, BMTB and FC correlated in 95% of cases (7/14). This study emphasizes that FC and BMTB are complementary in the diagnosis of acute leukemia and shows an excellent correlation rate between the two investigations.

MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIA WITH INV(3)(q21q26.2) OR T(3;3)(q21;q26.2) ABNORMALITY: A SINGLE CENTER EXPERIENCE

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Background. Recent World Health Classification (WHO) has incorporated acute myeloid leukemia (AML) with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) as a new entity in the category of AML with recurrent genetic abnormalities, that accounts for 1%-2% of AML. This type of AML can arise de novo or from pre-existing MDS. Prognosis of patients with MDS/AML with 3q abnormality (3q abn) is very poor with low complete remission (CR) rate and short long-term overall survival (OS) with standard chemotherapy. Patients and methods. We report baseline characteristics and outcome of 6 cases of patients with MDS or AML with 3q abn from our center. Demographics, blood counts, bone marrow features and evolution are shown in Table 1. Results. Four out of 6 pts were aged <65y. All of them received standard intensive chemother apy (IC). No complete remission was achieved followed by progression in all 4 cases. One pt underwent allogeneic stem cell transplant with subsequent early relapse. Other 2 pts (#5 and #6) have been recently diagnosed. Treatment received were non-intensive chemotherapy (NIC), one of them low-dose AraC and 5 azacitidine the other, carrying additional chromosomal abnormality (monosomy 7 in the context of monosom karyotype). The only treatment offering stable disease with OS and palliative therapy didn't improve overall survival (60 vs 56% at 12 months, p=N.S.) or progression-free survival (25% vs 24% at 12 months, p=N.S.). Conclusions. Although the decision (IC) of the patient's co-morbidities and performance status, we conclude that the outcome of aggressively treated patients is better, with prolonged overall survival and no significant increase in treatment-related complications. On the other hand, the use of consolidation didn't improve OS or DFS, in our series. While treatment decisions should be individualized to each patient, according to risk factors, performance status and co-morbidities, the recommendation to, whenever possible, enroll the patient in a clinical trial still prevails.
Conclusions. It is well known poor prognosis and decreased survival in patients with AML and genetic abnormalities on chromosome 3. Most are refractory to intensive chemotherapy and generally not well tolerated when dealing with elderly patients with frequent comorbidities. However, certain drugs as hypomethylating agents (5-azacitidine) have incorporated to treatment strategies after approval for low-blast count (20-30%) AML with promising results even in older AML pts when compared to conventional care regiments. Achievement of CR was not needed to extend survival in this pts. Whether this could be effective in this subset of pts with 3q abn must be addressed in larger prospective studies.

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WT-1 EXPRESSION LEVEL FOR MINIMAL RESIDUAL DISEASE MONITORING IN ACUTE MYELOID LEUKEMIA
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Monitoring of minimal residual disease in AML is an important part of patient management which potentially can increase overall survival by early detection of relapse. Many targets has been proposed for MRD monitoring in AML but all have known limitations as being useful only for a small subset of AML cases. WT-1 was shown to be overexpressed in most AML patients and is a useful target for MRD. Materials and methods: we used a previously described method which standardized the use of RT-PCR for WT-1 expression monitoring. A total 140 patient samples along with normal controls were analyzed. Samples were taken from 40 patients at presentation and different time point during follow-up. Results. Most of patients presented elevated levels of WT-1 expression at presentation (above 50 WT-1/10^9 ABL) ranging from 1 to 1.5*10^9 WT-1/10^9 ABL. During and after the therapy levels of WT-1 transcripts steadily decreased reaching normal levels. In 5 patients raise in WT-1 transcript level was predictive of a relapse as confirmed by fusion gene expression monitoring (4 cases with PML-RARA and 1 with AML-ETO). Conclusions. Due to expression of WT-1 in normal hematological tissue sensitivity of this assay is somewhat reduced, but in comparison of other methods of MRD monitoring it permits monitoring of the majority of AML patients regardless of other genetic abnormalities.

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AZACITIDINE FOR ACUTE MYELOID LEUKEMIA TREATMENT. ENCOURAGING RESULTS OF THREE SPANISH HOSPITALS
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Introduction. The potential and limitations of the standardized cyto- toxic chemotherapy (ARA plus Antracycline) for Acute Myeloid Leukemia (AML) have been assessed by different trials. In the search for novel treatment strategies, different trials have evaluated, with promising results, the role of Azacitidine (AZA), a DNA methyltransferase inhibitor, in the treatment of AML de novo or relapsed, before or after transplantation, or as maintenance after complete remission. Aims. To evaluate effectiveness and toxicity profile of Azacitidine in the treatment of AML patients. Methods. A retrospective study was carried out at 3 Spanish institutions including patients diagnosed with AML, defined as bone marrow blasts count >20%, receiving Azacitidine either as front line, salvage therapy or maintenance. Main points were progression free survival (PFS), overall survival (OS), complete response (CR) according to the IWG 2003 criteria; and toxicity, graded according to the CTCAE v3.0 of NCI criteria. Results. Data from 26 AML patients treated with AZA 75 mg/m2/day x 7 days every 28 days, were analyzed. Baseline characteristics were: mean age 67.6 years old (38-80), median 68y. Ratio male/female 17/9. Clinical history of previous Myelodysplastic Sym- drome (MDS) 54% (14/26). Effectiveness. AZA as front-line therapy: 13 patients, mean age 72.6y (62-80). Previous MDS 8/13 (62%) and poor prognosis cytogenetic 3/13. Five patients achieved cytological complete response (CR). At the time of report, with a mean follow up of 12 months (5-45), 8 patients are alive, 4 of them remain in CR, and 5 patients have died because of leukemia progression (mean OS 10.6 months), all of those with previous MDS. AZA as salvage therapy: 8 patients, mean age 63.6y (38-77). Previous MDS in 4 patients, mean of previous lines received: 1.5 (1-2). 2 patients achieved CR, one of them was consolidat- ed with allogenic transplant and died because of leukemia progression. At the time of report 1 patient is alive in CR with a follow up of 12 months, and 7 have died (OS 3.5 months). Hyperleucocytosis (>20,000/µL) presentation was correlated with a poor response rate and shorter OS; at the time of report there has been 4 deaths of 5 hyper- leucocytosis cases. On the other side, pancytopenic patients presented high response rate, often associated to a significant increase in platelet count, which allowed most patients to become transfusion-independent. AZA as maintenance after CR: 5 patients, mean age 61.1y (48-71). Previous MDS 2 patients. The mean of cycles received was 9.8 (2-21). With a mean follow up of 22.6 months (5.7-53.3), 3 patients are alive in CR, and 2 with an OS of 21-53 months. Treatment related toxicity was observed in all patients, neutropenia grade IV in 23 patients, febrile neu- tropenia in 12 patients and thrombocytopenia grade IV in 18 patients. All deaths were related to leukemic progression. No relevant extrahema- tologyc toxicity was reported. Conclusions. This study shows AZA is effective in AML and safe with no serious adverse event reported.

S-5AZACITIDINE IN REFRACTORY/RELAPSE OR UNFIT TO INTENSIVE CHEMOTHERAPY ACUTE MYELOID LEUKEMIA PATIENTS
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Background. 5-Azacytidine (AZA) prolongs survival in higher-risk MDS patients including those with 20-29% marrow blasts, currently diag- nosed as Acute myeloid leukemia (AML). However, no large AML series, especially with BM blasts >30%, treated with AZA have been reported. Patients and Methods. We have retrospectively analyzed results in patients having received at least 1 cycle of AZA in 7 centers for refractor- y/relapsed AML or not eligible for intensive chemotherapy (CT). 63 patients were included between Feb/07 to Feb/11. M/F: 42/21; median age 69 years (range 51-84). Median WBC was 2.2x10^9/L (0.1-17.5). Medi- an BM Blasts: 29% (20-94). 80 cases had >30% BM blasts. Karyotype (MRC classification), was adverse in 18 patients (including 5 Complex karyotype; 5 del5q; 6 -7delq; and 2 patients with 3q26). Intermediate in 57 (Normal karyotype 31, NPM1mut/FLT3wt in 4 and FLT3-ITD in 3 patients). Adverse in 23 cases (11(21); and 12(24)) and failed in 6 patients. Eighteen patients were treated for refractory/relapse AML after intensive CT or SCT (Auto-SCT=4; Allo-SCT=3); 22 had prior MDS y 28 as first line therapy. AZA doses were 75-100 mg/m2/day x 7 days (5-2). First evaluation was made after 4-5 cycles according to the Interna- tional Working Group criteria (AML-IWG-2006). Results. With a medi- an follow up of 9.5 months, patients had received a median of 5 AZA cycles (1-24). In first evaluation made after 4-5 cycles, an overall response rate (ORR) was observed in 17 evaluated patients (80%) including 9 CR (16%) and 8 PR (14%). Additionally, 16 (26.6%) patients achieved hema- tologic improvement (HI), according to MDS-IWG 2006 criteria. No pre- treatment characteristics as age, prior MDS, karyotype, BM blasts were statistically correlated with response. Eight responder patients pro- gressed within a median time of 8 months. The 12 mo Overall Survival (OS) was 40.9%, and 24 mo OS 15% and median OS of 11 months. In our series, pre-treatment characteristics as higher WBC (p=0.042), adverse vs intermediate cytogenetics (26% vs 54%, p=0.001) and >30% BM blasts (27% vs 50%, p=0.095) showed negative prognostic signifi- cance for 12 mo-OS. However, prior MDS (55% vs 31% 12 mo-OS, p=0,13) and disease status (AZA as first line or advanced disease: 42% vs 35%, p=0,5) did not influence OS. Achievement of ORR was associ- ated with improved OS (12 mo-OS 57% vs 35%, p=0,0013). In patients who received ≥3 cycles ≤21 days IWG response provided better survival (p=0.03). Conclusions. Patients diagnosed with relapsed AML and those not eligi- ble for intensive CI have limited treatment options. AZA can be a well-tolerated and effective alternative option in this group of patients. In our experience, these patients can achieve ORR of 90% with a 12 mo OS of 70%. Higher WBC counts, adverse karyotype and >30% BM blasts were associated with poorer OS but did not preclude response to AZA. Moreover, achievement of ORR or even HI is significantly associated with improved overall survival.
BACKGROUND. AML is a disease with marked heterogeneity in clinical and biologic features, response to therapy and survival. Despite major achievements in the treatment of AML, long term survival remains poor. About half of pediatric AML patients relapse and die internationally. There is no published data on pediatric AML in Lebanon. Aims: We aimed to identify clinical, cytogenetic, molecular findings and outcome data in the Lebanese population in comparison to international and regional data and to identify specific needs in order to improve outcomes. Methods: We underwent a retrospective chart review of children with AML diagnosed at 3 institutions in Lebanon in the past ten years. We filled and analyzed data collection sheets for each patient including demographic information, clinical, laboratory, molecular, therapeutic and outcome data. Results: We identified 24 patients with AML, 12 girls and 12 boys. Two had Fanconi anemia, one had Down syndrome, one had myelodysplastic syndrome with monosomy 7, and one had secondary AML after treatment for Burkitt lymphoma. Mean age was 8.6 years (range 1 to 24 years). Mean WBC at diagnosis was 66,500 (Range 2,100-376,000). Two were diagnosed as M0, 4 as M1, 2 as M2, 6 as M3, 5 as M4, 0 as M5, 2 as M6, and 1 as M7. Karyotype was normal in 37.5 % of cases. 25% had t(15;17), 4.1% had t(8;11), 8.3% had t(8;21), 8.3% had inv 16, 4.1% had t(9;11) and 4.1% had complex abnormalities. FLT3 was positive in 3 patients and was associated with high WBC at presentation and a poor outcome. NPM1 was tested in one patient and was negative. Death in induction was observed only in 3 patients with APML, hyperleucocytosis and bleeding and one child with M6 AML and Fanconi (16.6%). BM transplant was indicated in 17 pts and was performed in 8 pts in Europe or the United States: 1 had a cord blood transplant, 2 had a haploidentical BMT, 5 had matched related sibling BMT. Survival after transplant was 37.5%. Median survival for patients who died from disease progression was 25.8 months. Overall disease-free survival was 30.4% Summary/Conclusions: This is the first report looking at pediatric AML in Lebanon. Overall survival in this cohort was 30.4%. Areas of improvement would be initial support of patients with APML and hyperleucocytosis as high mortality was observed in these patients, and availability of BMT in a timely fashion for high risk patients. FLT3 positive patients died poorly with or without transplant. Further data collection to include the entire country is in process.

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OXIDATIVE STRESS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA EARLY STAGE. ASSESSMENT AND CORRELATION WITH PROGNOSTIC FACTORS

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Background. An important area of research of CLL is the identification of markers that are useful for predicting the likelihood of disease progression. Measurement of oxidative stress (OS) makes reference to the imbalance in favour of prooxidative state in front of antioxidative state. Aims. In a population diagnosed of early stage CLL and in a matched group control, we describe the values corresponding to different OS biomarkers. Its weight was set in a model of overall score (SOS) to assess the degree of OS in patients in early stages of CLL. Finally, we assessed whether there were differences in markers of OS in relation to different recognized prognostic factors. Methods. Informed consent was obtained. In a group of 57 patients, the values of different recognized prognostic factors were collected. In patients and in a matched group control, we determined the following markers of OS: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, reduced and oxidized glutathione, thio-barbituric acid reactive substances and global antioxidant capacity through the ORAC method. Then, we propose an SOS based on the analysis of these biomarkers. SOS was related with the presence of poor prognosis factors, in an attempt to introduce SOS as a prognostic marker of CLL early stage. Results. The SOS of patients did not follow a normal distribution. The score moved significantly towards more positive values, indicating OS (Figure 1). Higher oxidative state was evidenced through each patient’s biomarkers compared to control group (Table 1).

We found correlations between biomarkers of OS and analyzed prognostic markers. We created the variable ‘number of prognostic factors’
1158 PROGNOSTIC SIGNIFICANCE OF TELOMERE LENGTH IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN EARLY STAGE DISEASE

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Background. Chronic Lymphocytic Leukemia (CLL) is a genetically heterogeneous disease with a variable outcome. The identification of factors that could predict the clinical course of early-stage CLL represents a crucial objective in this malignancy. Although previous studies indicated that telomere length may be a useful independent prognostic factor in the risk stratification of CLL patients, limited information has been reported in asymptomatic early stage patients (Binet stage A). Aims. The present study was aimed at investigating the association of telomere length with the major biological and cytogenetic markers known to predict clinical outcome in CLL. The global DNA methylation levels of Alu and LINE sequences, was also investigated. Correlation with disease progression measured as the time elapsed from diagnosis to first treatment, was evaluated. Methods. We measured relative telomere length (RTL) by real-time PCR in a panel of highly purified (>90%) peripheral mononuclear CD19+ cells from 7 healthy donors and 77 untreated CLL patients. All cases were characterized by FISH for the most frequent chromosomal aberrations, namely trisomy 12 and 17p13.1, 11q22.3 and 13q14.3 deletions (Fabris et al. GGC, 2008). Molecular markers including mutation status of the heavy chain variable regions of immunoglobulin genes (IGHV), the expression of the 70-kd zeta-chain T-cell receptor-associated protein kinase (ZAP-70) and CD58 cell surface antigen protocols were previously reported (Cutrona et al. Haematologica, 2008). A quantitative bisulfite-PCR Pyrosequencing method was used to evaluate methylation of Alu and LINE-1. Results. We found a significantly lower RTL values in CLLs (median RTL=0.4 IQR 0.3-0.6) as compared with controls (median RTL=1.0 IQR 0.9-1.5) (P<0.001). A progressive and significant RTL decrease in low (1iq- and normal karyotype), intermediate (+12) and high (11q- and 17p-) cytogenetic risk categories (P for trend =0.008) was observed. Patients with IGHV mutated genes had longer telomeres than patients with unmutated genes (P<0.001). No significant association between telomere length and either CD38 or ZAP-70 expression was found. Telomere shortening was significantly correlated with hypomethylation of Alu (ρ=0.048) and LINE-1 (ρ=0.001), indicating a contribution to chromosome instability. Finally, follow-up analysis, available for 63 patients, showed a significantly higher risk of starting treatment for patients with shorter telomeres (P=0.037). Conclusions. Our results extended previous evidence that telomere length could be used as marker for the identification of CLLs with a different prognostic risk.

1159 MULTIPLE LIGATION-DEPENDENT PROBE AMPLIFICATION AND FLUORESCENCE IN SITU HYBRIDIZATION TO DETECT CHROMOSOMAL ABNORMALITIES IN CHRONIC LYMPHOCYTIC LEUKEMIA: A COMPARATIVE STUDY

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Background. Chronic Lymphocytic Leukemia (CLL) is a clinically heterogeneous disease characterized by overlapping combinations of prognostic significance. Although fluorescence in situ Hybridization (FISH) is the most common technique used to detect these abnormalities, it still remains a quite expensive time-consuming method. Aims. We aimed to evaluate the potential of the novel Multiplex Ligation-dependent Probe Amplification (MLPA) technique, to detect genomic alterations in CLL. Methods. Highly purified (>90%) peripheral mononuclear CD19+ cell populations from 100 untreated CLLs in early stage disease (Binet stage A) were included in this study. All samples were investigated by fluorescence in situ hybridization (FISH) for the presence of trisomy 12 and 17p13.1, 11q22.3 and 13q14.3 deletions. For MLPA analysis, DNA was prepared by means of 2 commercially available probes sets allowing the simultaneous screening of 56 genomic sequences. Results. Overall, a high degree of concordance (95%) between MFLA and FISH results was found provided that abnormal clone was present in more than 30% of leukemic cell population. The use of multiple MFLA probes allowed the fine the mapping of the 13q14 deletion and the identification of intragenic or small alterations undetected by FISH. Moreover, addition- al alterations in 2p24 (MYCN) (3pts), 8q24 (C-MYC) (1pt), 9p21 (K-ras) (1pt), 10q25 (PTPRC) (1pt), 11q22.3 (1pt), 13q14.3 (1pt), 17p13.1 (1pt) and 11q22.3 (1pt) regions not covered by a standard FISH assay were detected and all confirmed by FISH. Conclusions. Our data extend previous limited evidence that MLPA may represent a useful technique to characterize well-known lesions as well as to investigate additional genomic changes in CLL.
and lymphocyte count (R=471; p=0.04), β2-microglobulin serum level (R=0.470; p<0.001) and the percent of leukemic cells with the expression of ZAP-70 (R=0.456; p<0.001) and CD38 (R=0.274; p=0.004). The median treatment free survival (TFS) was significantly longer in patients with iNKT cell percentages higher than the median in the whole group (0.57%) as compared to patients with the lower percentages. Conclusions. Patients with CLL have lower percentages of Vα24 cells in peripheral blood as compared to age-matched healthy control. Moreover iNKT cell percentages decrease along with disease progression, correlate with CD4+ and CD8+ T cells and NK cells, and adversely correlate with Tregs as well as with negative prognostic factors. These results suggest an important role of iNKT cells in the development and progression of CLL, as well as their potential prognostic significance. Further studies involving larger groups of patients, and an assessment of iNKT cell function in patients with CLL are ongoing.

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TH17 CELL FREQUENCIES IN PERIPHERAL BLOOD OF PATIENTS WITH CLL ADVERSELY CORRELATE WITH T REGULATORY CELLS AND POOR PROGNOSTIC FACTORS

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Background and Aims. Th17 cells have been defined as a subset of CD4+ lymphocytes, characterized by their production of IL-17 and expression of the transcription factor RORγT. They have been implicated in inflammation and autoimmune diseases but still little is known about their prevalence and function in human cancer. In contrast to T regulatory cells, there are data showing both beneficial and harmful implication of Th17 cells in tumor development. In patients with chronic lymphocytic leukemia (CLL), many abnormalities in T cells populations have been described, but the data on TH17 cells are very limited. The aim of the study was to evaluate Th17 cells in peripheral blood (PB) of CLL patients and their correlations with the other populations of immune cells, such as T regulatory cells, NK cells, iNKT cells and prognostic factors. Methods: Frequencies of Th17 cells among CD3+CD4+ T cells, were measured in 56 patients with CLL, and 20 healthy individuals matched for age. Peripheral blood CD4+ T cells were analyzed for intracellular expression of IL-17A, FoxP3, IL-2, IL-4, IL-10, IFNγ and TNF using flow cytometry. Th17 cells were defined as CD3+CD4+CD25+FoxP3+, T regulatory cells (Tregs) as CD3+CD25+FoxP3+, NK cells as CD3-/CD56+CD16+ and iNKT cells were counted as CD3+ Vα24+ cells. Results. Analysis of intracellular expression of IL-17A in CD4+ T cells revealed significantly higher median percentage of Th17 cells in peripheral blood of CLL patients (18.4%) than in healthy donors (3.28%; p<0.001). Moreover, lower percentage of Th17 cells was observed in the age-matched healthy donors (R=0.326; p=0.004). In contrast, the percentage of regulatory T cells was significantly lower in patients (R=0.326; p=0.004) than in healthy donors (R=0.556; p=0.04), and inverse correlation between the percentage of Th17 cells and T regulatory cells (R=-0.228; p=0.04), CD34+CD4+IL-4+ (R=0.545; p=0.04) and CD35+CD4+TNF-1 cells (R=0.592; p=0.01). The percentage of Th17 cells inversely correlated with β2-microglobulin serum level (R=-0.292; p=0.04) and the percentage of leukemic cells with the expression of ZAP-70 (R=-0.244; p=0.04) and CD38 (R=-0.335; p=0.01). In patients requiring therapy during observation period, median percentage of Th17 cells was significantly lower comparing to untreated ones (5.74% vs 10.97%; p=0.02). Conclusions. Percentage of Th17 cells in peripheral blood of patients with CLL was significantly higher in age-matched healthy control, it decreased along with disease progression, correlated with iNKT cells, and adversely correlated with Tregs, CD4+ T cells expressing IL-4 and TNF as well as with negative prognostic factors. These preliminary results suggest that Th17 cells might be involved in CLL pathogenesis, and higher percentage in patients with early stage of the disease, may suggest that they take an important part in the control the leukemic cells growth, leading to faster disease of CLL. Further studies are required to confirm these observations.

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IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) WITH STEREOTYPED IGHV4-39,IGHD6-13/IGHJ5 REARRANGEMENT PROGRESSION MIGHT BE CONSIDERED AS RICHTER’S TRANSFORMATION INDEPENDENTLY OF HISTOLOGIC CONFIRMATION

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Background. Among factors predictive for CLL transformation to diffuse large B-cell lymphoma (DLBCL, RS), there is the usage of the IGHV4-39 gene with a stereotyped HCRDS belonging to subset 8 (according to Murray et al., 2008). Aims. This study describes 3 CLL cases carrying IGHV4-39 with stereotyped HCRDS-subset 8 in whom transformation presented with a sudden and impressive increase in peripheral B-cell count. Methods. Diagnosis of CLL was based on clonal lymphocytosis > 5.00 x10⁹/L (median lymphocyte count > 4.5 x10⁹/L), IGHV unmutated status, ZAP70 and CD38 expression was assessed by flow cytometry; FISH analysis was performed using commercially available probes (LSI13, LSI15SS19, CEP12, LSIp53, LSIATM, and BCL3 split signal probe [Dako]); cyto genetic analysis was performed after CpG-oligonucleotide DSF80 and IL2 stimulation. IGHV mutational status was evaluated according to IGMt. Results: Among 408 unselected CLL patients, 15 (3.7%) carried the IGHV4-39 gene and among them 3 showed the IGHV4-39/IGHD6-13/IGHJ5 rearrangement. In these pts a stereotyped HCRDS3-subset 8 was detected. No other pts displayed this specific stereotyped HCRDS3. At diagnosis, these 3 pts (all Binet stage A) showed similar biologic characteristics: IGHV unmutated status, ZAP70 and CD38 positivity, surface IgG (A light chain in 1 and k in 2), and isolated trisomy 12. Two pts carried BCL3 translocation, occurring as a consequence of t(14;19)(q32.3;q12.2). First-line treatment was administered at 20, 25 and 8 mos from diagnosis: pt 1 and 2 (age >70 yrs and comorbidities) received mono-chemotherapy and achieved a partial response, whereas pt 3 (a 63-year-old female) received a fludarabine-cyclophosphamide combination and achieved a complete response with low-level minimal residual disease. Transformation (at 65, 45, and 45 mos from diagnosis) was characterised by a sudden increase in lymphocyte count to > 150,000x10⁹/L after a period of stable disease. Morphologic evaluation revealed the predominance of large, lymphomase-like cells; enlargement of retroperitoneal lymph-nodes was detected in pt 2, but a biopsy could not be performed because of poor performance status. Splenomegaly was absent. LDH value was extremely high in all cases (from 6,300μmol/L to 42,900μmol/L). Bone marrow was extensively infiltrated by large B cells. Cytogenetic re-evaluation detected del17p in pt 1, no clonal evolution in pt 2 and a highly complex karyotype (defined as a3 abnormalities) in pt 3. CD23 expression was reduced in pt 2 and 3, while CD5 and CD20 expression was maintained. In all cases, analysis of IGHV-D-J rearrangement confirmed that B-cells were clonally related to the CLL phase. Pt 1 died 2 mos after transformation while on treatment with Alemtuzumab; pt 2 is still receiving treatment; pt 3 experienced an incredibly poor clinical course and died of respiratory failure caused by multiple pulmonary infiltrates in a few days after admission. Conclusions. (i) In our experience, HCD3-subset 8 stereotypy was associated with an aggressive outcome characterised by a predominant involvement of peripheral blood and bone marrow. Independently of a pathologically-proven shift to lymphoma, progression in pts with this peculiar HCD33 stereotypy might be considered as a transformation to Richter’s syndrome. (ii) In CLL the t(14;19) may cooperate with the stereotyped IGHV4-39/IGHD6-13/IGHJ5 rearrangement in causing aggressive evolution.
deletion was examined by FISH. All samples were analysed using two research assays based on an Affymetrix Microarray platform—(i) AmpliChip p53 Test (Roche) - a resequencing microarray designed for detection of single base substitutions and single base deletions in the coding and splice site regions of exons 2 - 11. (ii) CLL custom resequencing microarray (Affymetrix) containing probes for TP53 exons 2-11 and splice sites and for 9 other cancer-related genes; in particular, probes for 1 nt substitutions in the whole coding region and a probe for a common 2 nt deletion in TP53 (del 2N 209) were included. For discordant cases, the particular exon was analyzed by Sanger sequencing. Results. In all 12 samples previously assessed as wt-TP53 by FASAY, no mutation was found by either microarray assay. Across all three methods, 64 mutations were detected in 49 samples - 49 (76,6%) substitutions (i.e. 41 (63,1%) missense, 5 (7,7%) splice-site, 3 (4,6%) nonsense mutations), and 15 (23,4%) ins/del mutations (including 3 one nucleotide deletions). However, only 35 (54%) mutations out of 64 were recognized by all methodologies - 32 substitutions and only 3 ins/>1bp del mutations. Both microarray assays reached the same number of recognized mutations. Irrespective of the FASAY results, each microarray detected 47 mutations out of 64 (73,4%), 41 of which overlapped. Vice versa, 10 additional mutations identified by microarrays were not detected by FASAY (2 missense, 2 nonsense, 4 splice-site mutations, two 2 nt deletions), 4 of which were not possible to be detected by gDNA sequencing. Summary/Conclusions. Microarray resequencing methods are less time-consuming than classical sequencing. AmpliChip p53 Test is more user-friendly, while the CLL custom resequencing microarray enables parallel mutational analysis of multiple genes. All tested platforms may fail to detect mutations present in low percentage of cells (approx. 15-25%). Ins/>1bp del mutations and one nucleotide deletions may not be detected by all methods, suggesting that no single methodology currently used for TP53 mutational screening is coupled with mirna-650 expression

1164 THE RECEPTOR TYROSINE KINASE ROR1 IS EXPRESSED BY LYMPHOMA AND MYELOID HAEMATOLOGIC MALIGNANCIES

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Background. ROR1 is a member of the receptor tyrosine kinase (RTK) family and related to muscle specific kinase (MUSK) and Trk neutrophin receptors. It is considered a potent survival kinase, ROR1 is of importance during embryogenesis and organogenesis and is not expressed on normal adult lymphoid and non-lymphoid tissues. Our published data showed an ectopic expression of ROR1 in all chronic lymphocytic leukemia (CLL) cases. A recent publication has described expression of ROR1 in hematologic malignancies of lymphoid origin. In the present study we have investigated the surface protein expression of ROR1 molecule in a series of patients with lymphoid and myeloid malignancies. Aims. To study the expression pattern of ROR1 in hematological malignancies of lymphoid and myeloid origins using a ROR1 monoclonal antibody recognizing the extracellular domain of ROR1.

Methods. 137 cases belonging to different hematological malignancies including 35 cases of CLL, 8 cases of mantle cell lymphoma (MCL), 7 cases of marginal zone lymphoma (MZL), 11 cases of diffuse large B-cell lymphoma (DLBCL), 30 cases of follicular lymphoma (FL), 16 cases of acute lymphoblastic leukemia (ALL), 12 cases of acute myelogenous leukemia (AML), 9 cases of chronic myelogenous leukemia (CML) and 9 cases of multiple myeloma (MM) were analysed by flow cytometry. Ten aged-matched healthy donors PBMC and 10 reactive lymph nodes were used as controls. Results. Our results showed a statistically significant variation in the expression of ROR1 in various hematological malignancies as compared to controls (no expression of ROR1 in PBMC of healthy donors or reactive lymph nodes). In flow cytometry, 25/35 of CLL cases were positive for ROR1 surface expression range of 22-91%, 8/8 MCL (13-93%), 7/7 MZL (28-76%), 11/11 DLBCL (50-81%), 20/30 FL (12-89%), 12/16 ALL (11-83%), 9/12 AML (32-95%), 9/9 CML (57-80%) and 9/9 MM (42-85%). Conclusions. ROR1 is widely expressed at the protein level in most types of lymphoid and myeloid malignancies but not on normal blood cells. These results suggest that ROR1 may be a candidate molecule for targeted therapy in various types of hematological malignancies

1165 REARRANGEMENT OF µ IMMUNOGLOBULIN LIGHT CHAIN IN CLL IS COUPLED WITH MIRNA-650 EXPRESSION

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Background. MicroRNAs constitute 3-5% of predicted genes in human genomes, and are located in the introns (~60%) or exons (~10%) of protein-coding genes, or outside of known genes (~30%). Although their important role in the regulation of protein-coding mRNAs was described, very little is known about the actual regulation of their expression. Interestingly, the locus for immunoglobulin µ light chain (IgL) in humans includes an annotated miRNA gene miR-650 (encoded by 4 members of V2 subgenes for IgL) (Das, 2009). This could be of particular importance because it is known that the biology of CLL is driven by processes that depend on immunoglobulin structure and microRNA expression (Calin, 2005; Mraz, 2009). Moreover, microRNAs are physiologically involved in regulation of IgL production and V(D)J recombination (Vigotti, 2007; Koralov, 2008). Aims. The aim of this study was to characterize the expression and function of miR-650 in CLL. Methods. We have defined the immunoglobulin µ light chain rearrangement in a cohort of 40 CLL patients using BIOMED-2 protocol and the expression of surface immunoglobulins was verified by flow cytometry. The qRT-PCR (TaqMan Gene Expression) was used to study expression of miR-650 in this cohort. To assess the function of miR-650 cell lines (MEC-1, NALM-6) and CLL cells were electroporated with a RNA mimicking miR-650 (Dharmacon). This was followed by microarray expression profiling and western blot analysis of transfected cells revealed that the electroperoration with miR-650 led to a down-regulation of dozens of potential target miRNAs, and the validation of the putative targets is currently in progress. Conclusions.
We have shown that the expression of miR-650 is coupled with the regulation and usage of variable subgenes for lambda immunoglobulin light chain and this has potential relevance for B cell biology.

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### 1166

**CIRCULATING REGULATORY T-CELLS IN CLINICAL MONOCLONAL B-CELL LYMPHOCYTOSIS**

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**Aims**

To evaluate the number of circulating Tregs in patients diagnosed with clinical MBL and to compare it to clinical-biological features of the diseases.

**Methods.** Tregs number have been detected, by means of multicolor flow cytometry (CD45/CD4/CD25/CD127), in the peripheral blood of 56 patients with clinical MBL (30 M/26 F; mean age 66.5 ± 9.7 years; range 44 - 86 years), 74 patients with previously untreated CLL (56 M/38 F; mean age 68.7 ± 12.5; range 35 - 90 years) and 40 healthy subjects (20 M/F 20; mean age 55.8 ± 14.3 years; range 30 - 81 years).

**Results.** MBL patients showed a lower absolute number of Tregs (57.6/μL ± 38; range 4-154/μL), compared to CLL patients (70.7/μL ± 112; range 10-820/μL; p=0.004), but higher than controls (27.3/μL ± 10.9; range 5-49/μL) with an outstanding statistical significance. Noteworthy, there was not a higher number of CD4+/CD25+ cells within the CLL subset compared to MBL (p=0.16). No significant correlation was found between Tregs number and CD38+B-CLL patients showed a lower absolute number of Tregs (37.6/μL ± 10.9; range 5-49/μL) with a significance of increased number of human cells for 3/4 of sequentially tested samples (in one case only T-cell counts increased). Conclusions. The use of NOD/SCID IL2Rgamma(-/-) mouse strain led to 100% engraftment efficiency for MEC1 and CLL cells making it a promising tool for CLL xenotransplantation studies.


### 1167

**NOD/SCID IL2Rgamma(-/-) XENOGRAFT MODEL FOR CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background.** Monoclonal B-cell lymphocytosis (MBL) is a disease entity characterized by lower than 5000/μL circulating clonal B-cells in absence of other features of lymphoproliferative disorders. Regulatory T-cells (Tregs) constitute a small subset of cells involved in antitumour immunity and are generally increased in patients with chronic lymphocytic leukemia (CLL), for the diagnosis of which more than 5000/μL clonal B-cells in peripheral blood are required. Aims. To evaluate the number of circulating Tregs in patients diagnosed with clinical MBL and to compare it to clinical-biological features of the diseases.

**Methods.** Tregs number have been detected, by means of multicolor flow cytometry (CD45/CD4/CD25/CD127), in the peripheral blood of 56 patients with clinical MBL (30 M/26 F; mean age 66.5 ± 9.7 years; range 44 - 86 years), 74 patients with previously untreated CLL (56 M/38 F; mean age 68.7 ± 12.5; range 35 - 90 years) and 40 healthy subjects (20 M/F 20; mean age 55.8 ± 14.3 years; range 30 - 81 years).

**Results.** MBL patients showed a lower absolute number of Tregs (57.6/μL ± 38; range 4-154/μL), compared to CLL patients (70.7/μL ± 112; range 10-820/μL; p=0.004), but higher than controls (27.3/μL ± 10.9; range 5-49/μL) with a significant correlation was found between Tregs number and CD38+B-CLL patients showed a lower absolute number of Tregs (37.6/μL ± 10.9; range 5-49/μL) with a significance of increased number of human cells for 3/4 of sequentially tested samples (in one case only T-cell counts increased). Conclusions. The use of NOD/SCID IL2Rgamma(-/-) mouse strain led to 100% engraftment efficiency for MEC1 and CLL cells making it a promising tool for CLL xenotransplantation studies.


### 1168

**IGHV UNMUTATED CLL B CELLS ARE MORE PRONE TO SPONTANEOUS APOPTOSIS AND DEPEND FROM THE ANTI-APOPTOTIC EFFECT OF ENVIRONMENTAL SIGNALS**

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3Background. Chronic lymphocytic leukemia (CLL) is a clinically heterogeneous disease. Patients with unmutated (UM) tumor immunoglobulin heavy chain variable regions (IGHV) have a worse prognosis than patients with mutated (M) IGHV. The tumor microenvironment [cytokines (i.e. IL-4 and CD40L) bone marrow stromal cells (BMSC) and nurse-like cells (NLCs)] are important survival factors for CLL B cells. Aims. Aim of this study was to assess whether and to what extent UM and M CLL cells differently depend on the local microenvironment for their survival. Method. M and UM CLL cells negatively selected by magnetic beads isolation were cultured in the presence or in the absence of IL-4, CD40L, murine stromal cells (M2-10B4), NLCs, CLL-derived BMSC and autologous, negatively selected T lymphocytes. Apoptosis necrosis were evaluated by annexin V and propidium iodide (PI) staining. The intracellular expression of Bcl2 and the activity of NF-kB were evaluated by flow-cytometry and by EMSA, respectively. Quantitative analysis of ReA and RelB NF-kB subunits was performed with the TransAM Flexi NF-kB Family assay kit (Active Motif, USA). CK and chemokines (CC) were measured using a multiplex suspension array system (Bio-Rad Laboratories S.r.l) in culture supernatants. Results. Leukemic cells purified from the peripheral blood (PB) of 15 UM patients (UM CLL B cells) showed a significantly higher apoptotic rate in 7-day cultures as compared to M CLL B cells. In both M and UM CLL B cells, high basal levels of Bcl-2 expression and NF-kB activity were detected. On day 7, Bcl-
patients were classified as Binet A (63%). The comparative analysis of two CLL patients (23 female and 39 male) and 10 age-matched hematopoietic segregation and cytokinesis. The three major aurora kinases (AURKA, AURKB, and AURKC) have been previously described and they are addressed to cancer immunotherapy and no study has comprehensive expression leads to genetic instability and trigger the development of tumors. However, the majority of studies involving AURKA/B are expression and NF-κB activity were preserved when cells were cultured as UM unfractionated PB mononuclear cells (UM PBMC) as compared to purified UM CLL B cells (Fig. 1C).

This observation suggested the presence of a pro-survival element in the PB of these patients. NLCs were not responsible of this prosurvival effect since, unexpectedly, NLC were defectively generated from the PBMC of UM patients, whereas they were abundantly generated by the PBMC of M patients. In spite of the lack of generation of NLC, leukemic cells viability was very similar in the non adherent fraction of M and UM PBMC. Conversely, autologous T cells played an essential role in supporting UM CLL B cells survival. Indeed, a significant NK-f mediated prosurvival effect was observed when purified UM CLL B cells were cultured in the presence of autologous purified T cells (Fig. 1D). This prosurvival effect of circulating T cells was exerted both in cell-to-cell contact and in trans-well condition and was associated to increased secretions of TNF-α, PDGF-BB and IL-8. Conclusions. Despite their more aggressive features, UM CLL B cells are more susceptible to spontaneous apoptosis and their viability strictly depend from the presence of environmental prosurvival signals. This vulnerability of UM CLL B cells might be exploited as a selective target of therapeutic interventions.

GENE EXPRESSION PROFILE OF AURKA AND AURKB IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): CORRELATION WITH CLASSICAL CYTOGENETIC (GTC) AND HEMATOLOGICAL PARAMETERS

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Background. Aurora kinases are mitotic kinases especially important in the regulation of G2/M phase of the cell cycle and various mitotic events, including centrosomal duplication, mitotic spindle assembly, chromosome segregation and cytokinesis. The three major aurora kinases (AURKA, AURKB, and AURKC) have been previously described and they are related to different stages of mitosis. Furthermore, aurora kinase overexpression leads to genetic instability and trigger the development of tumors. However, the majority of studies involving AURKA/B are addressed to cancer immunotherapy and no study has comprehensively examined the role of these genes in leukemogenesis. AIMS. In the present investigation, we have evaluated AURKA and AURKB gene expression in peripheral blood (PB) cells of CLL patients, by real time quantitative PCR and correlate the findings with hematological parameters established for CLL and classical cytogenetics METHODS. Sixty two CLL patients (25 female and 39 male) and 10 age-matched hematologically healthy donors were selected for our study. The majority of the patients were classified as Binet A (63%). The comparative analysis of AURKB expression of leukemic and normal samples was calculated as a relative quantification to the GAPDH gene. Moreover, AURKA/B gene expression from leukemic samples was calculated as relative quantification to normal CD19+ cells from healthy donors and expressed as 2-ΔΔCt. The metaphase induction in CLL was performed by using the immunostimulatory method that employs the combination of DSP30 and IL-2. Chromosome preparations were obtained by using standard procedures and the subsequent cytogenetic analysis and interpretation were made according to the ISCN (2009). All cytogenetic and gene expression data (AURKA/B) were validated by FISH analysis with a specific set of probes. RESULTS. According to median value of AURKA/B expression, patients were divided into two groups (±3.4, considered as AURKA+ and >2.3, considered as AURKB+) and their clinical and biological characteristics were correlated.higher AURKA/B expression was observed in CLL samples compared with PB normal samples (AURKA [mean value of ΔCt ± SD]: 0.09±0.003 vs 0.07±0.001, p=0.02; AURKB: 0.166±0.01 vs 0.09±0.002, p=0.02). Moreover, AURKA/B patients presented a significantly high leukocyte count compared with AURKA+/B- patients (AURKA [WBC counts ± SD]: 27.8±5.5 vs 68.8±5.8, p=0.003; AURKB: 40±5.5 vs 66±6.2, p=0.0023, respectively). However, no significant differences were found regarding to Binet classification, gender or platelets count. In addition, Pearson correlation showed that there is a significant association between high expression of AURKA/B and complex karyotype (relative risk: 2.4 [95% CI. 1.46-3.92]; p<0.001). Among the classical cytogenetic profiles, the most frequent one, normal karyotype was found in 15 patients (24%) and metaphases with abnormal karyotype were seen in 47 subjects (76%). CONCLUSION. In this investigation we demonstrated a significant correlation among high expression levels of AURKA/B genes in CLL with chromosomal abnormalities and other hematological patients (32 female and 39 male) and 10 age-matched. Overexpression of aurora kinase genes have been extensively studied in solid tumors. In CLL this observation may be associated to the genesis of chromosomal abnormalities and possible be used to predict the course of genomic instability in CLL patients.

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PERSISTENT POLYCLONAL BINUCLEATED B-CELL LYMPHOCYTOYSIS (PPBL): IDENTIFICATION OF A SPECIFIC CYTOGENETIC PROFILE AND A LONG TERM FOLLOW UP OF 151 PATIENTS

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Background. PPBL is a rare disease characterized by a chronic, stable, persistent and polyclonal B-cell lymphocytosis, the presence of binucleated lymphocytes in the peripheral blood and a polyclonal increase in serum immunoglobulin-M. AIMS. We report here the cytogenetic characteristics and the long-term follow-up of 151 patients with PPBL. More concretely, we performed a cytogenetic study of 10 patients using the most resolutive cytogenetic method based on SNP array was performed in order to study more extensively cytogenetic abnormalities in PPBL. Methods. PPBL was diagnosed in 151 patients. Conventional cytogenetic analysis (CCA, n=139), fluorescent in situ hybridization (FISH, n=129) and SNP array were performed at diagnosis in the course of PPBL. We used Affymetrix® Cytogenetics Whole-Genome 2.7M Arrays® in 10 typical PPBL patients. The DNA was extracted from peripheral CD19- B-cells and CD19- cells purified using Miltenyi® technology (AutoMACS Pro Separator®). Results. PPBL was diagnosed in 26 male and 125 female patients, with a median age of 40 years (18.9-66.2). At diagnosis, supernumerary isochromosome 3q (+i(3)(q10)) was detected in 82/139 patients (59%) using CCA and FISH. FCC was detected as a sole abnormality in 7139 patients (5%). No abnormality was identified in 50/139 patients (36%). Chromosomal instability was observed in 77/139 patients (55%) at diagnosis and persisted during PPBL follow-up. SNP array analysis in 10 patients (5 male, 7 female). CD19- B-cells were separated by immunomagnetic cell sorting. We performed DNA arrays on CD19+ and CD19- cells in 7 patients, CD19+ cells in 2 patients and CD19- cells in 1 patient. Recurrent gene copy number (GCN) gains were detected on the long arm of chromosome 3q (3q) and identified only in leukemic cells. The size of GCN gains was variable between patients, from thirty kilobases to the whole 3q. GCN gains did not involve all the CD19- B-cells (mosaicism phenomenon). Non recent GCN aberrations involving the whole genome confirmed the presence of genetic

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instability in all patients. Out of the genes amplified on 3q of CD19+, cells, a higher frequency of FFS patients or complete amplification of one particular gene. After a median follow-up of 34 months (1-347), six cases of IgM MGUS were observed. Three patients developed solid cancers (2 pulmonary cancers and 1 cervical carcinoma). Three patients developed diffuse large B-cell lymphoma (DLBCL), two patients a splenic marginal zone lymphoma (SMZL). Conclusion. 3q has been reported to be involved in the progression of some cancers of the upper aerodigestive tract and cervical carcinomas. In addition to MGUS cases, we report here 5 cases of lymphomas developed during the course of PBPL. The clinical follow-up with genetic instability and the presence of recurrent cytogenetic abnormalities on 3q led us to consider PBPL as a premalignant state and require a carefully long-term follow-up of PBPL patients.

1171 MUTATIONAL STATUS AND GENE REPertoire OF IGHV-D-J REARRANGEMENTS IN SERBIAN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Background. The mutational status of immunoglobulin heavy variable region (IGHV) genes is the most stable molecular prognostic marker in chronic lymphocytic leukemia (CLL). It defines two CLL subtypes, mutated (M-CLL) and unmutated (U-CLL), with significantly different clinical behavior. U-CLL cases usually present a non-progressing disease and have considerably longer survival than unmutated cases, who tend to have a progressive disease. Bisected IGHV gene usage as well as in mutated vs. unmutated CLL cells, and the expression of almost identical, stereotyped heavy complementarity-determining region 3 (VH CDR3) sequences among some patients implies the role of specific antigens in the pathogenesis of the disease. Furthermore, there are population differences in mutational status and IGHV gene usage which may reflect different genetic background and/or effect of environmental factor. Aims. The aim of this study was to analyze the mutational status and IGHV gene repertoire in Serbian CLL patients. It included 85 CLL patients, 57.4% with progressive and 42.6% with non-progressive disease. Methods. IGHV-IGHD-IGHJ rearrangements were amplified by multiplex RT-PCR using 5’ primers specific for leader sequences of IGHV gene subgroups, in conjunction with 3’ primers complementary to IGHJ genes. Clonal RT-PCR products were sequenced and analyzed using the ImMunoGeneTics database. Results. Overall 88 alleles have been analyzed, since 3 patients (3.5%) expressed biallelic rearrangements, both of the same mutational status (mutated). We found that 56.8% of rearrangements carried mutated IGHV genes, whereas 43.2% carried unmutated IGHV genes. Among M-CLL patients, 44.7% presented a progressive disease, in contrast to 73.3% among U-CLL course patients. Comparison of FFS means revealed a significantly longer FFS in mutated cases (p=0.01). The most frequent IGHV subgroup was IGHV3 (55.7%) followed by IGHV1 (27.3%), IGHV4 (12.5%), IGHV5 (2.3%), IGHV2 (1.1%) and IGHV6 (1.1%). No IGHV7 subgroup members were identified. IGHV3 genes in mutated rearrangements (69.4% vs. 30.6%), in contrast to IGHV1 subgroup genes which were predominantly unmutated (70.8% vs. 29.2%). A total of 29 IGHV genes were identified, 7 of them accounting for 56.9% of all rearrangements. The most frequent were IGHV3-23 (14.8%), IGHV1-69 (10.2%), IGHV3-33 (8%), IGHV1-2 (6.8%), IGHV1-18 (6.7%), IGHV3-30 (5.7%) and IGHV3-44 (5.7%). GHDI genes showed the following distribution: GHDI3 - 38.4%, GHDI2 - 22.1%, GHDI6 - 12.8%, GHDI7 - 10.5%, GHDI4 - 8.1%, GHDI5 - 7% and GHDI7 - 1.2%. The most frequent IGHJ gene was IGHD4 (48.9%), followed by IGHD6 (28.4%), IGHD3 (11.4%) and IGHD5 (11.4%). No IGH1 and IGH2 genes were identified. IGHD4 was used preferentially in mutated (72.1%) and IGHD6 in unmutated rearrangements (80%). There was a significantly lower median VH CDR3 length in mutated (15 a.a.) vs. unmutated (21 a.a.) cases (p<0.001). Conclusions. Our study showed strong correlation between IGHV gene mutational status and clinical course of CLL. Progressive disease was observed predominantly in patients expressing unmutated rearrangements, while non-progressive disease predominated among M-CLL patients (p=0.026). Frequencies of IGHV subgroups and their mutational status resembled those observed in other Mediterranean countries, with exception of IGHV4 subgroup genes which were underrepresented in our cohort.

1172 THE INTERPLAY BETWEEN PKC α AND PKC β IN CLL

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Background. PKCα and PKCβII belong to a classical subfamily of serine/threonine protein kinases that are involved in regulation of proliferation, differentiation, cell migration and apoptosis in normal cells. These enzymes are structurally similar and are activated by phospholipids, diacglycerol (DAG) and Ca2+ binding. In a number of cancers including breast, prostate, brain and gastric cancer, PKCα has been implicated as a tumour promoter. However, in other cancers such as colon, thyroid, pituitary and pancreatic cancer, it serves a tumour suppressive role. Thus it is important to delineate the particular function of PKCα in specific cancers in order to better direct therapies. PKCβII plays an important role in regulating signals generated by the BCR, and has been shown to be overexpressed in CLL compared to normal mature B cells, correlating with a worse overall prognosis. In the current study, we explore the relationship between these two isoforms in CLL. Aims. Although the expression profile of PKCβII has been associated with a more aggressive CLL phenotype, its function in the generation and propagation of CLL remains largely unclear. Here we characterise the role of PKCα and PKCβII in CLL by investigating the signalling mechanisms associated with their expression. Methods. Murine CLL-like cells were generated from stem/progenitor cells of wild type ICR mice by retroviral introduction of PKCα and PKCβII construct, and subsequently maintained in an in vitro B-cell supportive system or injected into RAG-/- mice for in vivo studies. For translational significance, human CLL cells were isolated from peripheral blood of diagnosed patients and subsequently co-cultured in a microenvironment mimicking that of circulating and proliferative tumour cells. Results. Our studies indicate that PKCα acts as a tumour suppressor in CLL, and its subversion leads to an upregulation and sustained overexpression of the PKCβII isoform. Importantly in human CLL, we show a decrease in PKCα transcript and protein levels in more than half of CLL cases studied, complimented by an increase in PKCβII transcript and protein levels. In addition, the increase in PKCβII was consistently correlated to increases in tumour-associated ERK and mTor signalling. Finally we identify cyclin D1 to be overexpressed in our mouse model of CLL and in human CLL, which, alongside the implicated signalling pathways leads to cells that have a survival and proliferation advantage over normal B cells. Summary. Although structurally similar, PKCα and PKCβII serve very different and significant roles in CLL. The downregulation of tumour suppressor PKCα results in an upregulation of PKCβII coupled with increased oncogenic signals, contributing to the malignant phenotype of CLL.

1173 CD38 RS6449182 POLYMORPHISM IS ASSOCIATED WITH OVERALL SURVIVAL IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. CD38 antigen is well-established independent marker of poor prognosis in B-CLL. CD38 molecule is thought to be important in delivering of growth and survival signals in leukemic cells. The single nucleotide polymorphism 184C>G in the regulatory region of CD38 gene (CD38 rs6449182 SNP) has recently shown to affect CD38 expression profile of PKCα and PKCβII in human CLL, we show a decrease in PKCα transcript and protein levels in more than half of CLL cases studied, complimented by an increase in PKCβII transcript and protein levels. In addition, the increase in PKCβII was consistently correlated to increases in tumour-associated ERK and mTor signalling. Finally we identify cyclin D1 to be overexpressed in our mouse model of CLL and in human CLL, which, alongside the implicated signalling pathways leads to cells that have a survival and proliferation advantage over normal B cells. Summary. Although structurally similar, PKCα and PKCβII serve very different and significant roles in CLL. The downregulation of tumour suppressor PKCα results in an upregulation of PKCβII coupled with increased oncogenic signals, contributing to the malignant phenotype of CLL.

Conclusion. The interplay between PKCα and PKCβII in human CLL is an inherent component of the disease, and PKCβII plays a key pathogenetic role in the disease development and whether it provide prognostic information on the clinical outcome of the disease. Methods. PCR-RFLP analysis was used for CD38 rs6449182 genotyping: genomic DNA was amplified in PCR using primers designed by Aydin et al, 2008, with following enzymatic digestion of PCR products. The digested products were resolved on 3% agarose gel and analyzed. Results. SNP was studied in a total of 304 CLL patients and in the group of 217 control individuals. The median follow-up from diagnosis in CLL patients was 4.1 years (range 0.1-27
The majority of patients were at Binet A or B stage at diagnosis (45% and 40% correspondently), 70% cases were IgVH unmutated. We found that the frequency of the variant homozygous CD38 rs6449182 GG genotype was significantly higher in CLL group in comparison with control (14.8% vs 6.9%, P=0.012).

1175 ANALYSIS OF POTENTIALLY BICLONAL CLL PATIENTS WITH TWO OR THREE PRODUCTIVE IGH REARRANGEMENTS

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Background. Majority of chronic lymphocytic leukemia (CLL) patients develop a monoclonal disease and thus can be characterized by single immunoglobulin heavy chain gene (IGH) rearrangement. Interestingly, double productive IGH rearrangements can be sporadically detected with an incidence about 2-4% and can indicate simultaneous bicalonal/biphenootypic disease. Malignant subclone diversification due to BCR receptor editing or monoclonality with a lack of IGH allelic exclusion has been previously described as other possible reasons of this phenomenon. Here we present the detailed analysis of patients expressing two/three functional IGH rearrangements who were examined for IGHV mutational status. Aims. We performed a detailed characterization of selected CLL cases with double/triple productive rearrangements and followed their clonal evolution during course of the disease. Methods. B-lymphocytes were separated from peripheral blood using Ficoll-Faque PLUS (GE Healthcare) and RosetteSep Kits (StemCell). IGHV status was assessed according to the ERIC recommendations and Ig light chain (IGL) utilization was examined using cDNA as an input material. At DNA level, clonal IGH and IGL rearrangements were confirmed by BIOMED-2 protocol and also unproductive rearrangements were analyzed. Immunophenotypization was performed on separated CD19+ cells using panels of monoclonal antibodies including those against CD19/IgLambda/IgKappa, CD19/CD20/CD23, CD19/CD45/FMC7. Other prognostic markers, such as chromosomal alterations and TP53 mutational status, were available in all cases. Results. Double/triple productive IGH rearrangements were in total detected in 35 patients comprising 8.6% of our cohort. CLL patients expressing multiple in-frame IGH rearrangements, 12 of them (34%) were further analyzed in three subsequent samples per patient in average. At DNA level, all productive rearrangements were confirmed; out-frame IGL rearrangements were found in nine cases (75%). Immunophenotypization revealed two separated populations in five cases (42%) differing mainly in Kappa and Lambda IGL that corresponded with PCR results and were considered as true bicalonal cases. In one patient, bicalonality was presumed while two-frame IGL Lambda were present. Based on bioinformatics analysis, BCR editing was excluded in all 12 patients because no pair of IGH rearrangements shared D-J junction. Conclusion. Bicalonal CLL is a very infrequent phenomenon representing approximately 50% of cases with double productive IGH rearrangements. We performed particular characterization of 12 potentially bicalonal patients. Six of them were bicalonal at least in one time-point during their course of CLL according to immunophenotype and/or PCR. In several cases, proportion of particular populations changed in time. BCR editing was excluded in all these patients. We conclude that subsequent monitoring of CLL patients and repeated IGH rearrangement assessment is necessary for understanding of malignant disease evolution. Further analysis of these patients is in progress.


1174 IMPAIRED ANTIGEN PRESENTATION AND POTENT PHAGOCYTIC ACTIVITY IDENTIFYING TUMOR-TOLERANT HUMAN MONOCYTES: DEMONSTRATION IN ISOLATED CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Monocyte exposure to tumor induces a transient state in which these cells are refractory to further exposition to cancer. This phenomenon, termed “tumor tolerance”, is characterized by a decreased production of proinflammatory cytokines in response to tumors. Aims. Identify tumor tolerance phenomena in monocytes from Chronic Lymphocytic Leukemia patients and their relationship with prognostics markers. Methods. Samples of peripheral blood from CLL patients were processed for monocyte isolation and phagocytic activity was measured. Tumor lymphocytes were cocultured with control monocytes and induced tolerance status was measured. Results: We have established a human model of tumor tolerance and have observed a marked down-regulation of MHCII molecules as well as the MHCII master regulator, CIITA. These cells combine an impaired capability for antigen presentation with potent phagocytic activity. We also show that circulating monocytes, isolated from patients who suffer from mature B-cells neoplasms, share all the determinants that characterize cells locked in a tumor tolerant state. In addition, monocytes from these patients and supernatants from their culture induced a tumor tolerance state in controls monocytes. Conclusions. Monocytes from CLL patients are locked in a tumor tolerant state with a lower capability of antigen presentation and therefore an impaired immune response.
1176 GENOMIC ABNORMALITIES DETECTED BY MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) MAY PRECEDE THE CLINICAL ONSET OF CHRONIC LYMPHOCYTIC LEUKEMIA BY SEVERAL YEARS

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Background. Chronic lymphocytic leukemia (CLL), the most common leukemia in adults in Western countries (Linch, 1992), is a biologically heterogeneous disease with a variable clinical course and many aspects of the pathogenesis are awaiting final clarification. B lymphocyte monoclonal rearrangements may last for longer than 7 years before the disease becomes clinically detectable (Frezzato 2005, Landgren 2009) but the triggering events leading to the disease progression are largely unknown. No data are available on the time of the first onset of the cytogenetic abnormalities detected by Fluorescence In Situ Hybridization (FISH) in more than 50% of cases at diagnosis (Dohner, 2000). Aims. To identify genomic abnormalities in healthy subjects, enrolled in a prospective population-based investigation, who subsequently developed CLL. Methods. Six patients with a diagnosis of CLL, according to standard morphologic and immunophenotypic criteria (Cheson 1996) have been identified in a cohort of 14396 healthy subjects who had been enrolled during years 1993 - 1996 in an ongoing prospective clinical survey also providing DNA samples preservation. FISH analysis has been performed in all the patients as part of the diagnostic work-up for CLL. We performed Multiplex Ligation-dependent Probe Amplification (MLPA) analysis (an emerging new tool apt to detect some genomic abnormalities with high sensitivity and specificity) (Buui, 2006; Al Zaabi, 2010) in all these subjects as well, after an informed consent was obtained. If a chromosomal abnormality was detected (by FISH and/or MLPA) we performed MLPA on DNA preserved samples collected at the enrolment in the clinical survey, 54 to 89 months before diagnosis. Interphase FISH with SEC63 (6q21), C-MYC (8q24), ATM (11q22), GLI (12q13), DLEU1 (13q14 and p53 (17p13) probes was performed on peripheral blood samples according to the ISCN criteria (Brothman, 2009). DNA was analysed with MLPA P040 test kit (MRC Holland), including set probes for 11q25 (ATM), 12p12.3-12p13, 12q14-12q24.3, 13q14.2 (RB1), 13q4.3 (KCNQ4-ATP7B), 17p15.1 (p53) chromosomal regions, according to the manufacturer’s protocol. Results. FISH analysis at diagnosis showed a del 13q14 in 3 subjects (nuclear positive interphases: 90% in 2 cases; 10% in 1 case). MLPA result was consistent with FISH only in the two patients with 90% deletion. In one of them MLPA revealed the same deletion to be present 54 months before diagnosis (see Table for details). Conclusions. We firstly report a CLL-associated genetic deletion detected a long time before the clinical diagnosis. The deletion includes the DLEU1 locus, proposed as the most likely candidate tumor suppressor gene (Wolf, 2001; Ouillette, 2008). We have been able to evaluate only a few cases but our report could prompt future investigations on the role of different genetic abnormalities and their meaning in the pathogenesis of CLL.

1177 CHARACTERIZATION OF CLL CASES BY I-FISH, IGHV STATUS AND CD38 EXPRESSION: A SINGLE CENTER STUDY

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Background. Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a variable clinical course. Aims. Here we report the characterization of a cohort of 90 CLL patients from central Sardinia using an integrated diagnostic laboratory workflow including immunophenotyping, cytogenetics and IGHV mutation status. Methods. Ninety CLL patients (M 54, F 36, age 41-90) were diagnosed between 2007-2010 by standard criteria. FISH analysis on interphase nuclei with probes for the detection of trisomy 12, 11q22.3 (ATM), 13q14 (D13S319 and D13S319) and 17p13 (TF5S) was performed in peripheral blood cells. In a sample of 30 CLls, peripheral blood was incubated in presence of the immunostimulatory CpG-oligonucleotide DSP30/Interleukin-2 (IL2) and RNA was analyzed for IGHV mutation status following the Biomed-2 protocol. Sequence data were analyzed using the International Immunogenetics database (IMGT, http://imgt.cines.fr). The IGHV gene was assigned as mutated with a cut-off of 2%. Expression of CD38 was analyzed on FACS using conjugate antibodies (anti-CD38-FITC/anti-CD19-PE) and positivity was assigned with a cut-off of 20%. Results. All patients included in the study could be successfully analyzed by interphase FISH. Trisomy 12 was detected by FISH in 15.5% (14/90) patients, 13q14 deletion in 40/90 (44%), TF5S deletion in 12% (11/90), while the 11q deletion was observed in 7 patients (7.7%). Correlation between CD38 expression and FISH aberrations showed del13q14 in 40% of the CD38-negative CLls while TF5S was present in the 74% of the CD38-positive subgroup. The status of somatic mutations in the IGHV gene was available in 45 cases. Twenty-six (58%) displayed an unmutated IGHV status, while the remaining 19 cases (42%) were mutated IGHV gene. The IGHV gene family usage within the mutated subset was VH4>VHSVH2> VH1>VH7, whereas IGHV3 was the most frequently used in the unmutated group, being expressed in 13 patients. IGHV1-69 genes were present in 8 CLL patients overall (2 unmutated). Correlation between cytogenetics and mutation status showed that the poor prognosis marker TP53 deletion was present in 2/6 (33.3%) of unmutated CLls and in 2/19 (10%) of mutated cases. CD38 expression was detected in the 43% and 28% of the mutated and unmutated cases respectively, and showed a tendency to correlate with cytogenetic abnormalities. According to age, TP53 and 13q14 deletions (21% and 60%, respectively) were more frequently detected in CLL cases diagnosed before age 55 when compared to older patients. When clustering analysis was performed, only 8 out of 45 sequences were assigned to a specific subset according to Murray et al, while 13 sequences, all unmutated, clustered based on the pattern they shared following the TEIRESIAS algorithm. This cluster included stereotype rearrangements in the IGHV3-21, IGHV3-11 and IGHV1-69 with the longest HCDR3 sequences. Summary. We found that cytogenetic adverse prognostic markers were more frequently detected among younger CLL patients and the ratio of unmutated/mutated patients is higher than in other population. We have a low incidence of IGHV3-21 and IGHV1-69 in Sardinian CLls suggesting that the frequency of specific IGHV CLl may be related to geographic, ethnic, or environmental background.

1178 GENE EXPRESSION PROFILE IN PATIENTS WITH CLL TREATED WITH CLADRIBINE AND CYCLOPHOSPHAMIDE REGIMEN

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Background. An accumulation of CD5/CD19/CD23 positive cells in bone marrow and peripheral blood due to their resistance to apoptosis is a key event in chronic lymphocytic leukemia (CLL). Therefore, apoptotic gene expression could be essential to understanding the background of the disease. The microarray technique is one of the most innovative and powerful tools, providing a wealth of information on gene expression which can be helpful in identification of diagnostic and prognostic factors and than can lead to a selection of the most appropriate therapy. Cladribine and cyclophosphamide (CC) is a safe and very effective drug combination used in the first line therapy for CLL patients. Aims. The aim of this study was to investigate the gene expression profile in CLL cells with regard to factors involved in the apoptosis cascade in patients treated with CC protocol. Methods. The study involved 8 CLL patients who were previously untreated. All the patients were diagnosed and followed at the Department of Hematology, Medical University of Lodz, Poland, according to the IWCLL 2008 criteria. The study received the approval of The Ethical Committee of Medical University of Lodz (Dnr. RNN/196/07/KE). Informed consent was obtained from all the patients. Fresh blood samples were collected from all the enrolled patients before and after 2 weeks of the first cycle of CC treatment
(clarithromycin 0.12mg/kg - days 1-3, cyclophosphamide 600mg/m² - days 1-5; q 4 wks × 6 courses). Mononuclear cells (MNCs) were separated according to the standard protocol and used for further studies. The gene expression profiling was measured by means of microarray method (TaqMan Low Density Array, Human Apoptosis Panel). This microarray method was based on real-time PCR technique. Ninety six transcripts were placed on the array, 5 of which were endogenous controls. Measurements were conducted in duplicates. The relative expression of each gene was quantified by the comparative cycle threshold (Ct) method (ΔΔCt), using 18S as an endogenous control. Fold change (RQ) for each gene was evaluated. RQ is the differential expression after treatment to gene expression before treatment. For significant fold change the value of 2 or greater than 2 was chosen. Results. Data analysis highlighted 8 out of 93 examined apoptotic genes whose expression was significantly important. 5 genes (BIRC 1, BIRC5, BIRC8, CARD6, HRK) form a cluster by means of average linkage method. The most significant differences in gene expression before, against and after treatment are demonstrated by antiapoptotic genes such as: BIRC1, BIRC5, BIRC8 whose expression decreased. However, the expression of 5 proapoptotic genes such as: PUMA, CARD6, HRK, APAF1 and TNFRSF10B (TRAIL-R2) significantly increased. Conclusions. Our results show that the treatment with CC regimen leads to overexpression of genes involved in the intrinsic pathway of apoptosis. Nevertheless, further studies into the clinical usefulness of this observation for the development of new therapeutical strategies are vital.

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USEFUL OF MONOCYCLICAL AND POLYCYCLICAL SERUM FREE LIGHT CHAINS AS A PREDICTIVE BIOMARKER OF PROGRESSIVE DISEASE IN A SERIE OF B-CLL PATIENTS: EPIDEM STUDY

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Background. The B-CLL, is the most common type of leukemia in the West countries. Several immunophenotypic, genetic and molecular markers allow their inclusion in different prognostic groups, recently serum free light chain (FCL) quantitation have shown abnormalities in 39-54%, and this marker is associated with shorter time to treatment (TTT) and inferior overall survival (OS). Objectives. To determine FCL abnormalities in a prospective cohort of CLL patients (EPIDEM project) and explore the effects of both monoclonal and polyclonal FCL elevations in the outcome of CLL patients. Material and Methods. A prospective, descriptive, and analytical and cross section of incidental cases of B-CLL in Aragon, Navarra, Basque Country and Cantabria during the period: 1/09/2007- 31/08/2008. The study involved 13 hospitals that have collected the clinical characteristics at diagnosis and biological samples for DNA, RNA and serum extraction stored in the Biobank. The study has the approval of the Committee on clinical trials and investigation of Aragon (CEICA) and patients have signed IC. We designed a database for the study where the variables are collected: demographic, staging (Rai / Binet), lymphocyte count, immunophenotyping (IP) (CD38, Zap70), genetic testing (CG) and molecular (Ig VH genes mutation), serum earmark samples using the FreeLite FLC assay (The Binding Site, Ltd., Birmingham, UK). Elevated FLC was defined as either kappa or lambda reference range (k=19.4 mg/L, l=26.3 mg/L). Monoclonal FLC elevation was defined as elevated FLC with an abnormal FLC ratio. Polyclonal FLC elevation was defined as elevated FLC with a normal FLC ratio (0.26-1.65). Follow-up to progression, development of primary tumors and response to treatment were analyzed. Results. We collected data from a total of 96 new cases. The analysis includes 61 cases: men (H) 31 (50.8%) females (M) 31 (50.8%). Average age: 70.8 years (41-91) Males: 63.3 years and Females: 71.6 years. 52.4% were older than 70 years. Staging at diagnosis (Binet and Rai): A0 68.8%, 13.1% A1, All 3.2%, 1.6% B1, BI 8.1%, BII 3.2%, 3.2% CIV. 18.1% starts with splenomegaly. 29.5% had no lymph node involvement or extranodal affection. CG: del13q (18.0%), trisomy 12 (18.0%), del11p (6.5%), del17p (1.6%), normal (45.9%). IP: CD38 positive (22.9%), Zap70 positive (16.3%). Ig VH mutated (21.3%). Abnormalities in FLC were present in 48% of the CLL patients. In 35.0% elevated kappa or lambda was observed, 20.0% had a monoclonal FLC and 15.0% had a normal FLC. Monoclonal F (p<0.05) was associated with high risk features of CD38+, CD49D+, Zap70+, IGHV unmutated and having high risk FISH (del17p13; del11q22). Conclusions. The more common genetic aberrations were: del13q and trisomy 12. Normal karyotype type is related to early stages, absence of spleen enlargement and B symptoms, typical immunophenotype and predominance of Ig VH gene mutation and CD38+ and Zap70 negative. The analysis of FLC at diagnosis could be a predictive biomarker in CLL.

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MATURATION OF THE MIR15A/MIR-16-1 FAMILY IS IMPAIRED IN CHRONIC LYMPHOCYTIC LEUKEMIA

A role as tumor suppressor has been proposed for the miR15a/miR-16-1 family of miRNA genes localized in a critical region in 13q14 that is deleted in more than 50% of CLL patients. These two miRNA genes regulate cell cycle progression and apoptosis in cell lines and in vivo, and knockout of the syntenic region in mice results in the development of CLL-like lymphoproliferative disease. miR15a/miR-16-1 is maturation at the Drosha processing step that produces the pre-miRNA precursors, while a miRNA used as a control (miR-155) had a positive correlation with its primary transcript as expected. In addition, also when compared to CD19-sorted normal B-cells. Moreover, in CLL cells levels of miR-16 and miR15A were inversely correlated to their pri-miRNA transcripts, while a miRNA used as a control (miR-155) had a positive correlation with its primary transcript as expected. In addition, also the levels of precursor miRNA molecules of the 13q14 miRNA genes were low in CLL patients. This strongly suggested a defect of miRNA maturation at the Drosha processing step that produces the pre-miRNA from the pri-miRNA molecule. Therefore, we grouped patients according to the observed levels of pri-miRNA and pre-miRNA: CLL patients with a ratio of these two processing intermediates (pri-pre) above the average level observed in non-malignant CD19-B-cells were included in the high ratio group (58% of our cohort CLL patients), while the rest was grouped in the normal ratio group (42%). Patients with high pri-pre ratio had significantly lower mature miRNA levels compared to patients with normal ratio. In contrast, the respective ratio of the precursor molecules of miR-155 that were used as control did not differ in the two groups. These findings suggest that there is a processing defect that reduces maturation of the miR15A/miR-16 in a subset of CLL patients. In order to test the actual processing activity in CLL cells, we used a luciferase based in-vivo Drosha processing assay and could show a significant reduction of pri-16-1 processing in patients belonging to the “high pri-pre ratio” group. These findings underline the role of miR15A/miR-16 in the pathomechanism of CLL and show a complementary route of inactivation that suppresses genomic loss of the critical region in 13q14 and its transcriptional downregulation in CLL.

MEASUREMENT OF SERUM FREE LIGHT CHAIN (SFLC) IS A SIMPLE, COST EFFECTIVE AND POWERFUL TEST TO EVALUATE ADVERSE FORM OF CLL AT DIAGNOSIS AND TO FOLLOW THE RESPONSE TO TREATMENT

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Background. Previously published data have emphasized the utility of SFLC assessment in newly diagnosed CLL and have concluded that abnormal SFLC ratio was related to adverse prognostic factors and a shorter time to first treatment. Aims. The aims of our study were to confirm the utility of SFLC at CLL diagnosis in a prospective study, to compare with known prognostic factors, and to evaluate the interest of the
test in treated patients' follow-up. Methods. We investigated SFLC level and ratio in 63 patients newly diagnosed with CLL at our centre between August 2007 and December 2010. Blood lymphocytes immunophenotype systematically included CD3 for RMH scoring and surface light chain expression analysis. Prognostic factors such as Binet stage, lymphocyte doubling time, CD38 and ZAP-70 expression, FISH analysis for F53, ATM, or RB deletion and chromosome 12 number, serum thymidine kinase and serum soluble CD25 were also studied. Serum B2-microglobulin and immunoglobulin (G, M and A) levels were also measured. In the same period of time, SFLC were assessed before and after chemotherapy in 52 treated patients, allowing 121 control measurements (one to seven per patient), and were compared to clinical and haematological response and minimal residual disease (MRD) measured by 5-probe fluorescence in situ hybridization (FISH) and/or complete karyotype, according to recommendations. Results. Thirty patients (48%) had an abnormal SFLC level and/or ratio at diagnosis. The ratio kappa/lambda was increased in 19/21 cases (90%). No relation was found between the level of SFLC and white blood cell and/or blood lymphocytes number, neither with the level of light chain expression on B-lymphocytes as determined by immunophenotype. The type of free light chain was constantly the same as the one expressed at the B lymphocyte surface. Abnormal SFLC was associated with adverse prognostic factors such as advanced Binet stage at diagnosis (B and C, p < 0.001), short lymphocyte doubling time (LDT) (R<0.0001), ZAP-70 positivity (p < 0.05), adverse cytogenetics (F53 deletion or trisomy, p<0.01). No relation was found with the presence of a serum monoclonal immunoglobulin peak, which was detected in 3 patients only. However a decrease in normal immunoglobulin serum level was evidenced in 22 of the 30 patients with abnormal SFLC (73%), with a significant correlation (p = 0.001). Analysis of long term free survival (TFS) duration between patients with abnormal SFLC and first line treatment showed a relation to SFLC. Multivariate analysis confirmed that short LDT, ZAP-70 expression, and F53 or ATM deletion were independent risk factors in Binet stage A CLL, and that abnormal SFLC was a high independent risk factor. Abnormal SFLC CLL had shorter TFS (HR = 27, 95% CI = 4.15-175.5), confirming that the disease was more aggressive in this group. Median duration of patients’ follow-up after treatment, abnormal SFLC were associated with a positive MRD (p<0.0001), with a specificity of 86% and a positive predictive value of 96%. Conclusions. SFLC assessment is a simple, immediate and cost-effective test to appreciate the aggressiveness of CLL at diagnosis and to follow the response to treatment.

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RITUXIMAB-BASED CHEMOTHERAPY FOR AUTOIMMUNE CYTOPENIA OF CHRONIC LYMPHOCYTIC LEUKAEMIA
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Background. Chronic lymphocytic leukaemia (CLL) is characterized by an acquired immune defect that can cause autoimmune complications, including anemia (AIHA) and thrombocytopenia (ITP). AIHA occurs in 10% of advanced stage CLL patients; ITP occurs in 2-3% in early stage disease. CLL may be a presenting manifestation. There are limited effective treatment options for steroid refractory autoimmune cytopenias. Rituximab, an active agent against B cell malignancies, has also been noted to be active in certain autoimmune hematologic disorders. The purpose of this study was to evaluate the safety and efficacy of Rituximab-based chemotherapy in this complication of CLL. Patients and Methods. This prospective study examines the outcome in CLL patients with autoimmune phenomena (AIHA, Evans’ syndrome). Five patients were treated at our institution. Two patients who had previously been treated with alkylation agents (CHOP/CVP), one of them had received Fludarabine, two patients were newly diagnosed and had not received treatment. Treatment regimen (RCD): Rituximab was given at a dose of 375mg/m² intravenously (i.v) on day 1 (D-1). Dexamethasone 40mg i.v. D-1. Cyclophosphamide at a dose of 1000mg (total dose) i.v D-1. The treatment was repeated every 2 weeks of a total of six cycles. Results. Median age was 65 years (range 44-74) and there were five male patients. All of them had Binet stage C disease, and ECOG Performance status was 1. Response in autoimmune cytopenias was evaluated by frequent blood counts and Coombs test. 4 of 5 patients achieved a remission of their cytopenia. One of them is still received the treatment. Median pretreatment hemoglobin was 6.7 g/dl and post-treatment hemoglobin was 12.8 g/dl. The mean pretreatment platelet was 147 G/L and post treatment was 213 G/L. Three patients converted to Coombs negative platelet after RCD. Median duration of response was 14 months. The patients were evaluable for toxicity; grade 4 toxicity none was noted in one patient and needed supportive care by haemopoitic growth factors. One patient died of progressive disease (CLL) 06 months after the response to RCD therapy. Conclusions. Autoimmune phenomena, largely related to blood cells, are based in the immune dysregulation of CLL. Our results indicate that a rituximab-based combination regimen (RCD) is highly effective in treating this complication of CLL, and show a safety profile.

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ASSOCIATION OF HEMATOLOGICAL NEOPLASIAS AND MERKEL CELL CARCINOMA
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Background. Merkel cell carcinoma (MCC) is a rare and aggressive neuro-epithelial skin tumor which has been reported to occur in association with other primary neoplasms, including haematological malignancies. Immune suppression and dysregulation are well established risk factors for the development of MCC and may provide the explanation for the reported association of this tumor with neoplasms of the immune sys-
with relapsed/refractory indolent or mantle cell lymphomas. Aims: The purpose of this study was to analyze data from the Israel Cancer Registry (ICR) relating to the epidemiological characteristics and the incidence of second cancers in patients with MCC, with special emphasis on haematologic malignancies and especially chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). Methods: Examination of the ICR records revealed 305 cases of MCC diagnosed in Israel during the period 1982 and 2009. Data were collected on age, gender and ethnic origin, dates of diagnosis of MCC and other tumors, as well as causes and dates of death when applicable. The incidence of second neoplasms in MCC was compared to those recorded in 4446 age-ethnic and period matched controls diagnosed with primary neoplasms in the Jewish population. Age specific standardized incidence ratio(SIR) was calculated with a 95% confidential interval (CI). Results: Incidence of MCC increased during the time periods reported. 97 % of MCC cases occurred in Jewish Israelis, while the remaining 3 % included Arabs and non- Arabs non- Jews. Median age at diagnosis in the Jewish patients was 73.3 years and 56 years in the Arab population respectively. One hundred and four patients (34%) had a second neoplasm, 73 evident before, and 31 after the diagnosis of MCC. The SIR for non- haematologic malignancies did not show a higher proportion of second tumors among MCC patients. Thirty of the 104 second cancers (28.8%) were haematological malignancies and of these 23 were detected before and 7 after the diagnosis of MCC. The SIR for second haematologic malignancy was 5.89 for males (95 % CI: range; 1.55-5.23) and 3.05 for females (95 % CI: range; 1.06-5.04). The most frequent haematologic neoplasias recorded were SLL/CLL (46.6%) and lymphoma (80%). There were also two cases of myeloproliferative diseases and single patients with multiple myeloma, hairy cell leukemia, myelodysplastic syndrome (MDS), mycosis fungoides. Conclusions: There is a high incidence of second haematologic neoplasias in Israeli Jews with MCC and these are mostly CLL and lymphomas. These results are in accordance with some reports from other countries showing a high prevalence of MCC, especially in patients with CLL and lymphoma. This rare but significant association should be taken into consideration when evaluating patients with B-cell lymphoproliferative disorders and a co-existent skin tumor.

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CLINICAL EXPERIENCE OF BENDAMUSTINE-RITUXIMAB TREATMENT FOR RELAPSED INDOLENT NHL AND CLL

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Background. Promising results have been reported in studies evaluating the combination of Bendamustine and Rituximab (B-R) in patients with relapsed/refractory indolent or mantle cell lymphomas. Patients and methods: we have analysed the role of the combination B-R in 8 patients since January 2009. They received Rituximab 375mg/m2 (day 1) plus Bendamustine 90-100 mg/m2 (days 1+2) every 28 days for a maximum of 6 cycles. The median patient age was 69.9 years (56-86 range). Most patients were in advanced stages (II-IV). Histologies were distributed: MALT NHL 10 %, grade 2 follicular NHL 10%; mantle cell NHL 50%, CLL/lymphocytic lymphoma 50%. Patients were heavily treated with a median of 3 prior regimens, including anthracycline containing chemotherapy (n= 4) and purine analog chemotherapy (n= 7). All patients had received previously rituximab. Results. Of the 10 patients, 2 are going in the treatment and they have not yet evaluated, 1 patient died at 8 cycles to infection, and 1 patient was lost follow-up. A median number of 4.5 cycles was given (0-6 range). At the time of analysis November 2011, the median observation time was 10 months (2-22). Overall response 100% rate for patients treated with B-R. CR rate was 42.9% and 57.1% PR. Two patients in PR progressed at 9 and 10 months after they completed therapy. 1 patient in PR progressed, after 3 cycles. Haematologic toxicities were observed for neutropenia grade 5+. Patients were admitted in hospital in 22% cycles of bendamustine (Table 1). The B-R regimen was good tolerated by the patients, as evidenced by a lower rate of alopecia, number of infectious complications and esotomatis. We observed drug-associated erythematous skin reaction ( rash) in one patient. There is not association between prior purine analog chemotherapy. Conclusions: the combination of Bendamustine +Rituximab have an excellent tolerability profile, and CR rate in heavily treated patients with relapsed/refractory indolent or mantle cell lymphomas. Further follow-up will determine whether the high RC/RCu rate corresponds to prolonged PFS. Additional updates on response will be available at the time of presentation.

Table 1. Causes of admission in patients.
It is necessary to note that in 3 (60%) patients with complete clinical and morphological remission, molecular remission developed too. The results of the study have shown that a response on alemtuzumab therapy depends on the duration of this therapy and the spread of the tumor process. Conclusions. Monoclonal anti-CD52 antibodies are high effective among CLL patients that are refractory to fludarabine-containing chemotherapy regimens, and with the relapses of the disease, but toxicity was considerable.

**1187**

THE INTERNATIONAL ON-LINE REGISTRY OF RARE, CUTANEOUS AND CNS LYMPHOMAS OF CHILDHOOD

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Background. Certain types of lymphomas (follicular lymphoma, small cell lymphoma, marginal zone lymphoma, mantle zone lymphoma etc.) are rare in childhood. Also, CNS lymphomas and skin lymphomas are also relatively rare in childhood. Because of this rare occurrence the knowledge of clinico-pathologic correlations and prognostic factors is low worldwide. Aims. The project objective is to study clinico-pathologic correlations and analyse prognostic factors in rare lymphomas, CNS lymphomas and skin lymphomas in children (i. e. in the fragile population), further standardisation of therapy protocols, which will mean a benefits in the health care in Oncology and Pediatrics. Owing to the fact of rarity/low incidence of such lymphomas even in specialised centres there is a necessity of not only interinstitutional, but also international cooperation. Methods. Starting international database/registry in Brno (under auspices of I-BFM) is being designed as a virtual site which will collect all diagnostic and clinical information by means of filling the on-line accessible (but secured by codes) forms, placed on the web site of the database/registry. A part of the database/registry will be freely accessible www atlas of microscopic/histologic images (on-line virtual microscope). Data will be acquired inter-institutionally and internationally by means of filling of secured web forms (initial data will be put along with sending a case by cooperating institution, follow-up data will be added prospectively. Number of cases will depend on amount of these rare diagnoses in cooperating institutions. After collection of statistically significant amount of cases from cooperating institutions there will be statistic analysis of clinico-pathologic correlations and analysis of prognostic factors. Standardisation of therapy protocols will be performed. Summary/conclusions. The project will enable a study of prognostic factors, clinico-pathological correlations and standardisation of therapy protocols in rare lymphomas of childhood. Data will be acquired inter-institutionally and internationally by means of filling of secured web forms. Clinico-pathologic correlations and therapy responses will be analysed by standardised statistical methods. Prognostic markers will be analysed mostly by means of detection of expression of those potential markers by immunohistochimical antibodies which will work on lent formalin fixed paraffin embedded tissue material (paraffin tissue blocks), then after semiquantitative evaluation of presence/absence of expression will be statistically analysed with aim to find possible prognostic correlations. Information about cases will be supported by web atlas. The atlas will contain free online accessible high resolution diagnostic images of pathology of every case with short English description (high resolution virtual microscope) which can help to histopathologists in making the diagnoses. Results. After collecting significant amount of cases from cooperatin institutions there will be statistic analysis of clinico-pathologic correlations and analysis of prognostic factors. Standardisation of therapy protocols will be performed. Summary/conclusions. The project will enable a study of prognostic factors, clinico-pathological correlations and standardisation of therapy protocols in rare lymphomas of childhood. Data will be acquired inter-institutionally and internationally by means of filling of secured web forms (initial data will be put along with sending a case by cooperating institution, follow-up data will be added prospectively. Number of cases will depend on amount of these rare diagnoses in cooperating institutions. After collection of statistically significant amount of cases from cooperating institutions there will be statistic analysis of clinico-pathologic correlations and analysis of prognostic factors. Standardisation of therapy protocols will be performed. Summary/conclusions. The project will enable a study of clinico-pathologic correlations and standardisation of therapy protocols in rare lymphomas, CNS lymphomas and skin lymphomas in children (i. e. in the fragile population) by means of interinstitutional/international database/registry shared on secured web site (www.rarelymphomas.eu).

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EFFICACY, TOLERABILITY, COST-SAVING OF FRONTLINE ORAL FLUDARABINE AND CYCLOPHOSPHAMIDE COMBINATION THERAPY FOR CHRONIC B-CELL LYMPHATIC LEUKAEMIA AND LOW GRADE NON HODGKIN LYMPHOMA ELDERLY PATIENTS

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Background. The treatment decision of elderly patients has to be made individuating, not only the stage and risk factors of the disease, but also patients’ physical condition and social environment. Fludarabine was the first purine analogue with an oral formulation available for clinical use. The oral formulation offers equivalent efficacy and an improved tolerability profile compared to the IV formulation. IV Fludarabine requires several administrations that will expose patients to the risk of IV injection complications and the cost of trip to the hospital. Aims. We would like to show that frontline oral fludarabine and cyclophosphamide combination therapy, for B-cell lymphatic leukaemia and low grade non Hodgkin lymphoma aged patients, is well tolerated, efficacy and cost-saving. Methods. Between April 2006 and December 2010, 26 elderly patients (median age 75, range 69-82) were treated with treatment requiring B-cell lymphatic leukaemia (according to ESMO guidelines working group) and 10 elderly indolent stage a3 lymphoma non Hodgkin untreated patients (median age 74, range 59-80) received therapy with low dose of oral fludarabine (25mg/mq/die) and cyclophosphamide (150mg/mq/die) (FC) both from days 1 to 5 in once a week representation. Study duration consisted at 4 weeks intervals in outpatient regimen. Patients received antibiotic prophylaxis with trimethoprim/sulphamethoxazole (160/800 mg twice a day, 3 days a week) and allopurinol (300 mg once a day from days 0 to 4). Performance status was WHO 7 2 in all patients. Comorbidities, which included diabetes, hypertension and chronic heart disease, were present in 12 patients. The mean of administered cycles was 4 with range 2-6. No patients reduced dose and number of cycle because of haematologic and extra-haematologic toxicities. Specifically only 2 patients experienced grade III neutropenia, treated with GCSF. Results. Of definition of response was reviewed according to the updated IWCLL-NOE 2008 criteria and clinical criteria. Survival in overall patients was 100% and in subgroup FC patients was 90%. We obtained a response with an overall response 85%. All responder patients are alive and maintained response after mean follow-up of 20 months (range 2-44). We used Genzyme sponsored Excel program to compare direct hospital cost of oral to IV FC (both 3 days regimen). IV treatment required 18 day hospital accesses with total cost of €7.527, oral treatment was administrated subcutaneously, in standard dose, as first line therapy (2 patients), as consolidation for previously treated responsive patients (9 patients), as continuous treatment (2 patients, as consolidation for previously treated responsive patients (9 patients), as for refractory/ progressive disease (33 patients). 15 patients were checked for del 17p (3 patients del17p+), CMV reactivation was confirmed in 8 patients (2 deaths with encephalitis). 19 patients died; 2 patients were checked for del17p (3 patients del17p+), CMV reactivation was confirmed in 8 patients (2 deaths with encephalitis). Performance status was WHO 7 2 in all patients. Comorbidities, which included diabetes, hypertension and chronic heart disease, were present in 12 patients. The mean of administered cycles was 4 with range 2-6. No patients reduced dose and number of cycle because of haematologic and extra-haematologic toxicities. Specifically only 2 patients experienced grade III neutropenia, treated with GCSF. Results. Of definition of response was reviewed according to the updated IWCLL-NOE 2008 criteria and clinical criteria. Survival in overall patients was 100% and in subgroup FC patients was 90%. We obtained a response with an overall response 85%. All responder patients are alive and maintained response after mean follow-up of 20 months (range 2-44). We used Genzyme sponsored Excel program to compare direct hospital cost of oral to IV FC (both 3 days regimen). IV treatment required 18 day hospital accesses with total cost of €7.527, oral treatment was administrated subcutaneously, in standard dose, as first line therapy (2 patients), as consolidation for previously treated responsive patients (9 patients), as for refractory/ progressive disease (33 patients). 15 patients were checked for del 17p (3 patients del17p+), CMV reactivation was confirmed in 8 patients (2 deaths with encephalitis). 19 patients died; 2 patients were checked for del17p (3 patients del17p+), CMV reactivation was confirmed in 8 patients (2 deaths with encephalitis).
INCIDENCE OF ADVERSE EFFECTS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA TREATED WITH FC VERSUS FCR

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Background. CLL is the most common form of adult leukemia, accounting for 25%. CLL is a slowly unfolding from year to decades. Treatment of FC was and still is widely used in the treatment of first line in patients with CLL, with good results. However, introduction of Rituximab treatment in CLL patients has resulted in better outcomes and especially lower adverse reactions. Material and Methods. Between January 2008 - January 2011 we evaluated 62 patients with CLL in different stages of disease, of which 46 have followed treatment of FC and rituximab was introduced at 14. Of these, 24 were stage A, 6 stage B and 32 stage C, 32 were men, 30 women. All six cycles were performed either FC or FCR. Results: Blood complications occurred in 35 patients, 26 with FC (56.5%), 9 FCR (64.2%). Of these, neutropenia appeared in 20 patients treated with FC (43.5%) and 6 with FCR (42.8%), anemia in 15 patients treated with FC (32.6%) and 4 with FCR (26.8%), thrombocytopenia in 11 patients with FC (23.91%) and 3 with FCR (21.4%). Infectious complications occurred in 10 patients treated with FC (21.7%) and 2 with FCR (14.3%), most viral infections (herpes, EBV). Gastrointestinal side effects such as nausea and vomiting occurred in 2 patients, one from each group, one from FC treated group (7.14%). Asthenia and fatigue, due in part to anemia were reported in 40% of patients with FC and 45% of those with FCR treatment. Alopecia with marked psychological impact was seen in 15 patients (29.26%) of the first group and six in the second (42.8%). Conclusions. The data reported in our study are comparable with the literature, not statistically significant differences between the two groups.

CLINICO-BIOLOGICAL FEATURES FEMALES AND MALES WITH B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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B-cell chronic lymphocytic leukemia (B-CLL), the most common leukemia in the Western world is characterized by quite variable clinical course, which have been correlated with some clinical and biological markers. Among proposed prognostic factors the significance of gender is unclear, although in published trials females survive longer. A group of 286 unselected patients with B-cell chronic lymphocytic leukemia were studied for clinical and biological features. Sample analysis included CD38 and ZAP-70 stainings, fluorescence in situ hybridization (FISH) for chromosomes 11, 12, 13 and 17, level of bFGF, VEGF and TNF. There were 168 men and 118 woman. The sex ratio was 1.41. The median age at presentation was similar, for men was 64.0 (range 34.0-83.0) and woman 64.5 (range 30.0-86.0) p=0.8207. Patients were staged at diagnosis according to the Rai classification. The distribution of patients with Rai stage eg. low-risk (stage 0), intermediate-risk (stage 1 and 2) or high-risk (stage 3 and 4) was: 28.5%, 50.8% 20.4% for female and 22.6%, 54.2%, 23.2% for males respectively. There was significant lower median rate of hemoglobin and higher rate of thrombocytes and percentage of CD3+ cells in females than males with B-CLL (12.6±3.1 96 vs 13.1±2.4, p=0.0045; 195.44±68.33 vs 170.36±0.65, p=0.0007; 16.02±3.41 vs 13.15±2.13, p=0.0369). Overall, the median survival of female patients was 56.91 months compared to 50.49 months for males (p=0.1076). Treatment-free survival was better for females than males (median time to treatment 17.55 months versus 9.27 months, p=0.0208). All group of studied patients received different kind of chemotherapy as front line treatment (chlorambucil alone, chlorambucil and prednisone, fludarabine containing regiments, COP and CHOP regiments); 63.6% females and 75.4% males had required treatment. There were significant differences in how females and males were treated (p=0.0255): 31.3% females and 28.4% males received chlorambucil alone or chlorambucil and prednisone, 8.6% females and 16.6% males were treated with COP or CHOP, and 33.7% females and 30.4% males achieved fludarabine containing regiments. 48.6% females and 46.0% males achieved complete response after front line treatment, in 22.2% females and 16.8% males partial response and in 12.5% females and 15.1% males progression disease was observed. There was no significant correlation between percentage of CD38 and ZAP-70 cells, percentage cells with genetic abnormalities, level of bFGF, VEGF, TNF and gender of patients with B-CLL.

PROGNOSIS OF RELAPSED CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) AFTER COMBINED FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB (FCR) TREATMENT

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Background. FCR regimen, as the first-line therapy in CLL patients, results in high response rates, improved progression free survival (IFS) and overall survival (OS). However, more than 30% of patients relapse or have disease progression in 3-4 years after. An appropriate therapy for these patients still has not been determined. Aims. The aim of our study was to analyze further course of the disease in relapsed CLL patients (pts) after FCR treatment. Methods. 39 CLL patients relapsed after FCR, followed in our center from January 1995 to January 2011, were included in this retrospective analysis. We identified 2 main subgroups - relapsed patients after FCR regimen given as the first-line therapy (25pts, Group A) and patients who had been given FCR as the second-line (or more) therapy (16pts, Group B). Results. Groups’ characteristics were as follows: Group A, a median age at the start of FCR was 62 years (49-74; median FCR cycles: 5). Rai stage before therapy: Rai I n=4 (17%), Rai II n=6 (26%), Rai III n=5 (22%), and Rai IV n=6 (35%). Regarding new prognostic factors, unmutated IgVH gene was detected in 91%, mutat- ed in 9% of patients. A certain evolution in cytogenetic aberrations was observed in pts pre- and post FCR treatment: normal karyotype (4 vs. 6pts), del(13q) (5 vs. 5pts), del(11q) (7 vs. 5pts), trisomy 12 (2 vs. 2pts.) and del(17p) (1 vs. 3pts). In terms of response to therapy, 14pts (61%) achieved complete remission (CR), 7 (30%) partial remission (PR) and 2pts (9%) had progressive disease (PD). The same analysis was performed in the Group B. A median age was 64.5 years (38-78; median FCR cycles: 8). Median number of previous treatments was 1 (range 1-5), 7pts were treated with fludarabine. Rai stage before the start of FCR: Rai I n=2 (3%), Rai II n=4 (25%), Rai III n=5 (81%), and Rai IV n=5 (51%). Unmutated IgVH gene was detected in 58%, mutated in 42% of patients. Cytogenetic abnormalities: normal karyotype (7 vs. 5pts), deletion of 13q (5 vs. 4), deletion of 11q (2 vs. 2), trisomy of 12 (2 vs. 0) and deletion of 17p (0 vs. 2). Nine patients (56%) achieved CR, 3pts (19%) PR, 1pt (6%) had stable disease and 3pts (19%) PD. Median DFS1 (after FCR treatment) was similar in both groups: 11 (A) vs. 12 months (B), p=0.59, PFS2 (after second-line therapy) was 10.4 (A) vs. 5 months (B), p=0.03. Median OS calculated from the time of diagnosis was 85.8 (A) vs. 100.9 months (B), p=0.08 (Figure). Conclusions. Compared with other treatment options, FCR is superior in CLL patients regardless being administered as a first-line therapy or a treatment of relapsed CLL, however increased number of poor prognosis cytogenetic aberrations is observed after FCR regimen in relapsed patients. Prognosis of CLL patients relapsed after FCR appears to be very poor irrespective of the type of subsequent therapy.

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RITUXIMAB THERAPY IN PATIENTS WITH EXTRA NODAL MANIFESTATIONS OF CHRONIC LYMPHOCYTIC LEUKAEMIA

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Chronic lymphocytic leukaemia (CLL) is a common adult leukaemia, occurring in patients over 50 years of age with median age at diagnosis of around 65 years. While it remains an incurable disease, its indolent nature results in a varied prognosis with a median survival of greater than 10 years reported in early stage disease. Treatment is typically initiated when patients become symptomatic including alkalizing agents (chlorambucil or cyclophosphamide) but combination chemotherapy with vincristine, prednisolone or fludarabine may be required in some patients. Rituximab, a chimeric anti-CD20 monoclonal antibody, has shown significant activity in a variety of B-cell lymphoproliferative disorders. The CD20 antigen is also found on B-cells in CLL and rituximab therapy has shown clinical benefit in these patients. Extra nodal infiltration by small lymphocytes in patients with CLL is an uncommon manifestation. There is limited data on specific therapeutic strategies for treating these patients. The following case reports highlight the usefulness of rituximab in CLL patients with extranodal infiltration, with minimal additional use of chemotherapy. We present seven patients with various extranodal manifestations including 3 patients with cutaneous lesions with one of them showing amyloid deposits; 2 patients with renal involvement including minimal change glomerulonephritis (MN) and membraan GN; 2 patients with pulmonary infiltration. All these patients received a short course of chlorambucil therapy followed by rituximab therapy with significant improvement. The therapy consisted of weekly infusions at 375 mg/m2 followed by monthly infusions for 4 months. Some of the patients have required maintenance rituximab therapy. Follow up period range: 2-8 years (median 4 years). 5 patients are alive and well and 2 patients died of unrelated causes. The surviving patients have not required any additional therapy to treat CLL or extranodal manifestations. Hence, rituximab can be used as a single agent therapy in these patients.

CLONAL DIVERSITY IN CONCOMITANT MULTIPLE MYELOMA AND CHRONIC LYMPHOCYTIC LEUKEMIA CASES DEMONSTRATED BY IMMUNOPHENOTYPIC, FISH AND WHOLE GENOME SNP ANALYSIS

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The occurrence of multiple myeloma (MM) and chronic lymphocytic leukaemia (CLL) in the same patient is rare. We present three such cases with documented clonal diversity by flow cytometry, FISH and molecular analyses. Informed consent was obtained for all studies. Case 1 is a 54 year old female diagnosed originally with CLL who presented with bone pain. On FISH analysis two distinct cell types were noted - small cells having +12 and plasma cells (PCs) showing 3-4 copies of FGF, D12Z1, CCND1, ATM, TP53 and MYB. Marrow showed a restricted plasma cell with aberrant CD117 and CD19, CD5, CD23 + a restricted B-cells. Whole genome SNP analysis (Infinium System) confirmed dual clonal disease. Case 2 is a 68 year old male diagnosed with CLL 2 years previously. Marrow showed pancytopenia with increased PCs showing a restriction. Lymph node showed lambda restricted CD19, CD5, CD23 + B-cells. CLL FISH probes were negative. IEF showed three M components - IgM, IgG kappa and IgG lambda. Case 3 is a 27 year old female diagnosed with MM. Flow cytometric analysis performed on marrow showed kappa restricted PCs with aberrant CD117 expression. FISH analysis two morphologically distinct cell types: cells with smaller nuclei had an extra copy of 1q and loss of 13q(14) cells with larger nuclei appeared tetraploid with 4 copies of 1p and 6 copies of FGF3, CCND1, TP53 and BCR and 5-6 copies of IGH. Genome SNP analysis is pending. The results in all cases support distinct clonal evolution of these two B-cell neoplasms.

CORRELATION OF BCL2 GENE EXPRESSION AND BCL2/BAX RATIO WITH CLINICAL AND IMMUNOPHENOTYPIC CHARACTERISTICS OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. B-cell chronic lymphocytic leukemia (B-CLL) is a lymphoproliferative disorder characterized by accumulation of clonal B-lymphocytes, mainly due to aberrant apoptosis, which regulates cell aging and is known to be overexpressed in B-CLL. The activity of this protein is opposed by Bax, a homologous protein that accelerates the rate of cell death. B-lymphocytes Bcl-2 and Bax protein levels were found to be significantly altered in B-CLL. The mutational status of immunoglobulin heavy chain variable region (IGHV) divides B-CLL into two prognostic groups, depending on the presence or absence of somatic hypermutation, where unmutated IGHV genes are associated with considerably worse prognosis than mutated IGHV. Aims. We analyzed clinical characteristics, immunophenotypic profile, IGHV mutational status, Bcl2 and Bax gene expression in 53 patients with B-CLL. METHODS. Thirteen patients with relapsed/refractory CLL were included in this study. RESULTS. The expression of Bcl2 and Bax genes was measured in the bone marrow aspirates using real-time qPCR. The correlation between clinical parameters, surface antigen expression Bcl2 and Bax gene expression ratio in patients with B-CLL was assessed using Pearson correlation coefficient. All patients signed informed consent according to Declaration of Helsinki.

PILOT STUDY OF THE COMBINATION OF RITUXIMAB, IFOSFAMIDE AND FLUDARABINE (R-IFLU) IN RELAPSED/REFRACTORY CHRONIC LYMPHOCYTIC LEUKEMIA

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Background. Fludarabine given with cyclophosphamide and rituximab is considered the cornerstone of chronic lymphocytic leukemia (CLL) treatment. While several purine-analogs have been evaluated in combinatorial trial, no other member of the oxazaphosphorines family has been investigated in this setting. Ifosfamide has shown significant activity in non-Hodgkin lymphomas, but has been not yet evaluated in CLL. Aims. We conducted a pilot phase II study of ifosfamide given in combination with Fludarabine and rituximab (R-IFLU) in relapsed/refractory CLL patients. METHODS. Thirteen patients with relapsed/refractory CLL were enrolled. Therapy consisted of ifosfamide (750 mg/m2) and fludarabine (25 mg/m2) for three consecutive days (D1-3), and rituximab (575 mg/m2) on D3. Treatment was administered every 21 days up to 6 cycles. Response was assessed using the NCI-WG 1996 criteria. Maintenance with monthly rituximab (575 mg/m2) infusion was administered in responders for a total of 4 months. RESULTS. Median patient age was 63 years (range 51-71). The median WBC count was 61,800/mmc (3,160-122,000), and 9 patients were in advanced Binet stage (B and C). Six (46%) patients presented with bulky lymph nodes (>5 cm). Five patients (38%) showed an unfavorable cytogenetic profile, including 4 patients with del(11q) and 1 patient carrying del(17p). The median number of previous lines of therapy was 2 (1-4); all the patients were previously exposed to chlorambucil (69% being refractory) and 29% to Fludarabine (54% being refractory). A median of 4 cycles of therapy was administered (2-6); the overall response rate (ORR) was 69%, including 29% of CR. After a median follow-up of 4 years all the patients progressed, with a median progression free survival of 20.5 months (95% CI 8.1-40.1). Median overall survival was 47.6 months (95% CI 33.8-86.6). Hematologic toxicities were frequent: grade 3-4 neutropenia, thrombocytopenia and anaemia were reported in 69%, 15% and 8% of the patients, respectively. Infusion related toxicities occurred in 8 patients (62%) during therapy, a grade 3-4 event was reported in one patient. Conclusions. This pilot study shows that combination of ifosfamide, fludarabine and rituximab is feasible and effective in relapsed/refractory CLL. Further studies are needed to evaluate the potential role of ifosfamide as alternative oxazaphosphorine drug in those patients only partially responding to cyclophosphamide-containing regimen.
Results. High expression level of CD23 surface antigen correlates with shorter PFS (p=0.04), higher level of bone marrow infiltration (p=0.053), shorter lymphocyte doubling time (p=0.004) and advanced Rai clinical stage (p=0.014). The expression of CD38 surface antigen correlates with shorter PFS (p=0.008). High level of Bcl2 gene expression and Bcl2/Bax ratio correlates with shorter lymphocyte doubling time and shorter PFS. In the group of patients with mutated IGHV (p=0.01; p=0.004). High level of Bcl2 expression correlates with the following immunophenotypic profile: low expression level of FMC7 (p=0.02) and high expression level of CD23 (p=0.032) in all patients. High Bcl2 expression correlates with lower FMC7 expression level in the group of patients with unmutated IGHV (p=0.005). High Bcl2 expression correlates with Bax expression, nor with Bcl2/Bax ratio in this study. Conclusions. Our analyze showed that B-CLL with high CD23 surface antigen expression have more aggressive course with shorter progression free survival. Higher Bcl2 gene expression and Bcl2/Bax ratio correlate with the shorter progression free survival in B-CLL patients with mutated IGHV. We suggest that B-CLL with high level of Bcl2 gene expression is characterized by distinct immunophenotypic profile with increased CD23 and reduced FMC7 surface antigen expression.

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CLL REGISTRY IN ROMANIA - A PRELIMINARY REPORT OF THE ROMANIAN INITIATIVE GROUP IN DIAGNOSIS OF CLL (RGIDCLL)

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Background. Chronic lymphocytic leukemia (CLL), the most frequent chronic lymphoproliferative disorder, mainly in elderly people seems to have real incidence higher than in reports. The diagnosis based especially on flowcytometry, and molecular and genetics changes should define the prognosis subgroups of CLL. Aims. The present study done by the Romanian Group of Initiative in Diagnosis of CLL proposes to introduce flowcytometry in current screening of patients with lymphocytosis suspects in Romania. At the same time, to answer the real incidence of CLL in Romania based on the same panel and protocol concerning the recommended ESMO guidelines. Methods. We have analyzed 214 patients suspected with CLL in last year in few centers in Romania. Clinical assessment was done as the guidelines recommend- in all patients. The samples for immunophenotyping were collected from peripheral blood and sent from the centers to the central laboratory. The diagnosis was made by flowcytometry on a BD FACS Flow Calibur, with classical association of markers CD19, CD20, CD5, CD23, CD79b. In these patients we have analyzed other surface markers, like CD43, CD38, and intracellular markers ZAP-70. P53 mutation was done by FISH in a central laboratory, too. Statistical analysis with SPSS software was used to find correlations between clinical, immunophenotypical and genetic parameters, and therapy, too. Results. The patients with CLL were stratified by Rai clinical stage as follows: 17.89% stage 0, 16.58% stage I, 39.15% stage II, 10.67% stage III, and 16.80% stage IV. Positive diagnosis for CLL was found in 146 patients, represented 68% from the suspected cases. Negative cases represent other lymphoproliferative disorders and reactive lymphocytosis. 11 cases (5%) were diagnosed as monoclonal B-cell lymphocytosis. A presumpti- ve incidence was calculated from the regions which were involved in subjects recruitment. We found an incidence of 4.0559 · 100,000 people correlated to 3.476.357 people, higher than the incidence reported in other regions of world, and with a higher number of high risk (p53 positive) cases. It could be a high risk of this region related to the nuclear incidents from 80's involved in this pattern or not? We consider that this preliminary data are very useful to assess the real incidence and ethiologic factors of CLL occurrence in Romania. Funding: thanks to Roche-Hoffman Romania and Genzyme Romania for their support.

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RITUXIMAB IN COMBINATION WITH HIGH DOSE METHYLPREDNISOLONZE FOR THE TREATMENT OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA AND P53 DELETION

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Chronic lymphocytic leukemia (CLL) patients with del(17p) or del p53 do not respond to conventional treatment and have shorter survival. Studies have demonstrated that high dose methylprednisolone (HDMP) either alone or in combination with Rituximab is effective in such patients. The purpose. We evaluated the efficacy and complications of the combination of high dose methylprednisolone (HDMP) with the anti-CD20 monoclonal antibody, rituximab (R-HDMP) in heavily pre-treated CLL patients with del p53. Patients and Methods. We retrospectively studied 9 patients with CLL, (4 men and 5 women), who had del p53. The median age was 72 years (range 59-82 y). Two patients had Rai stage I, one patient Rai stage III and the remainder 6 patients were Rai stage IV. Four patients had bulky disease. All 9 patients had previously received other chemotherapy regimens (2-7 prior therapies). HDMP was given intravenously at a dose of 1 gm daily for 5 days in combination with rituximab 375mg/m2 on day 1 of a 28 day cycle for 6 cycles. They also received trimethoprim-sulfa, valacyclovir and fluconazole as prophylaxis during therapy and for two months after its completion. Results. Eight out of nine patients developed side effects (hyperglycemia: 3 patients, edema of lower extremities: 3 patients, increase of infections: 4 patients, osteoporotic fracture: 1 patient and myocarditis: 1 patient). Four out of 9 patients (44.48%) achieved partial response (PR), 2/9 patients (22.23%) had steady disease (SD), and 3/9 patients (33.33%) had progressive disease (PD). With a median follow up of 5 months all patients with PR had progression of the disease and one of them died, while all patients with SD and PD died. Conclusions. Although the number of patients is small, the results indicate that the combination of high dose methylprednisolone (HDMP) with rituximab (R-HDMP) is an active regimen in a poor prognostic patient group like heavily pre-treated CLL patients with del p53. Further evaluation in controlled trials is needed.

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LYMPHOCYTE CELL POPULATION DATA PROVIDED BY UNICEL DXH 800 IMPROVE IDENTIFICATION AND CLASSIFICATION OF LYMPHOPROLIFERATIVE DISORDERS

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Background. Lymphoproliferative disorders (LD) and B cell chronic lymphocytic leukemia (B-CLL), are heterogeneous disease whose diagnosis is based on automated WBC count, microscopy and immunophenotyp- ing. Our previous experience with VCS technology of Beckman Coulter showed important correlation between VCS Cell Population Data (CPD) and other data coming from microscopy, flow-cytometry, cytogenetics, and molecular biology.1 Since all these test are still part of the routine in clinical practice for B-CLL and LD diagnosis and classification, we decided to further explore UniCel DxH800 CPD could provide the same useful information to laboratory and clinical hematology.3 Methods: 16 patients samples (7 of them, untreated CLL) and 50 healthy donors were analyzed in this study with UniCel DxH800 that
performs leukocytes differential with the Flow Cytometric Digital Morphology (FCDM) technology, based on the measurements of Volume (V), Conductivity (C) and 5-angle Scatter light laser (UMALS, MAL5, LMALS, LALS, AL2) on cells in their native state. Mean (M) and standard deviation (SD) of FCDM measurements are collected in 56 CPD. Diagnosis of CLL and LD are based the WHO classification criteria. Results. Reference interval for lymphocyte CPD were calculated and compared with pathological samples’ ones. We confirmed our previous data showing the statistically difference (p<0.05) of mean volume LY (MV-LY) in CLL samples versus normal samples (83 a.u. vs 89 a.u.); SD-V-LY was 18.3 in CLL and 14 in normals. We discovered that axial-light-loss (M-AL2-LY) is also lower (41 vs 68). These CPD were able to describe the morphological findings of both clonal lymphocyte populations with low homogenous volume and CLL, with heterogeneous features. Even in some leukemic lymphomas we found correlation between MV-LY (95), AL2-LY (74) and morphological abnormalities of lymphoma cells. As an example we found a 3-years old ALL sample with lymphoctytosis and without leukocytosis in which the value of SD-LY-AL2 (14) vs normal (10), induced us to follow-up in the diagnosis. Scatter light CPDs seem to be also useful in differentiating abnormal cells and therefore they need to be investigated.5. Conclusion. In this study we presented some considerations on the useful information to laboratory and clinical hematology provided by UniCel DxH800 CPD. Different patterns of scatterplot with different CPD values can be the basis for the validation of the technology in large laboratory hematology lab. The first ones need screening tools while the second ones need classification tools useful also in the follow-up of the patients’ therapy and prognosis. These preliminary observations are now under investigation in a multicentric evaluation.

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BORTEZOMIB ENHANCES THE SENSITIVITY OF IMATINIB TO K562/ G01 CELLS
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Objectives. To explore the effect of proteasome inhibitor Bortezomib (BOR) on the sensitivity of imatinib to K562/G01 cell (imatinib-resistant chronic myeloid leukemia cell line) and the mechanism. MTT assay was used to observe the effect of growth inhibitory of cells. flow cytometry was used to detect cell cycle. Real time-PCR was performed to detect expression of COX-2 and MDR-1 mRNA. Results. Combined with 10-20nmol/L BOR could significantly enhance the sensitivity of drug, the reverse fold respectively was 1.83 and 2.72. G2/M phase cell cycle arrest could be seen by flow cytometry with BOR. K562/G01 cell over-expression of COX-2 and MDR-1, BOR could down-regulate COX-2 and MDR-1 expression. Conclusion BOR can enhance the sensitivity of imatinib to K562/G01 cell, the mechanism may be related to cell cycle G2/M phase arrest and down-regulating the expression of COX-2 and MDR-1.

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STUDY OF THE ASSOCIATION OF CYP2D6*4 POLYMORPHISM WITH THE RISK OF CHRONIC MYELOID LEUKEMIA
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Background. PCYP2D6 is a member of cytochrome P450 enzymes family which are involved in the detoxification of a wide range of xenobiotics and drugs. Several genetic polymorphisms had been shown to affect its activity which may results in increased susceptibility to malignant disorders. Aim of the Study: This study aimed to determine whether any association exists between genetic polymorphism in CYP2D6*4 and risk of chronic myeloid leukemia (CML). Subjects and Methods: Our study groups consisted of 50 CML patients and 40 unrelated healthy volunteers as a control group. Results. The frequencies of EM genotype (wild type) were 64% and 95% in CML patients and control groups, respectively. The frequencies of polymorphic IM genotype (homozygous variant) were found to be 26% in CML patients and 5% in healthy controls. The PM genotype (homozygous variant) was 8% in CML and not observed in control group. There was a statistical significant correlation between the CYP2D6*4 gene polymorphism and chronic myeloid leukemia patients with the frequencies of IM and PM CYP2D6*4 were higher in CML cases compared to controls (26% versus 5%, 8% versus 0%, p=0.004), on contrary, the frequency of EM was higher in controls cases compared to CML patients (95% versus 64%). As an estimate for the risk factor, the odds ratio (OR) of CYP2D6*4 polymorphism was 10.698 with a 95% confidence interval of 8.597-13.189. Conclusion: These data indicate a higher risk for CML in individuals carrying the IM and PM CYP2D6*4 and reflect the major role of environmental factors in CML pathogenesis. The present study establish significant association of CYP2D6*4 polymorphism with CML.

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EXPRESSION OF PHOSPHORYLATED STATS IN CHRONIC MYELOID LEUKEMIA: RELATION TO DISEASE STAGES
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Background. Chronic myeloid leukemia (CML) is characterized by the presence of the Ph chromosome (BCR/ABL chimeric gene) in hematopoietic stem cells. Clinically, it is manifested in three distinct phases: chronic, accelerated, and blastic. Signal transducer and activator of transcription (STAT) proteins are known to be regulated by cytokine receptors and are critical for driving transcription necessary for growth, survival, and differentiation of hematopoietic cells. BCR-ABL expression is constitutively activation of STAT5 and essentially bypasses cytokine or growth factor-dependent activation of STAT5. Aim: The aim of our work was to investigate the state of STAT5 phosphorylation in relation to CML disease stages as a possible indicator of BCR/ABL tyrosine kinase activity. Methods: The study was conducted on 39 CML patients including 17 male and 22 female with an age range of 19-79, a mean of 59±15 and a median of 41.5 years. Patients were diagnosed as CML and stages defined according to the WHO classification of myeloid neoplasms. Twenty two patients were in chronic phase (group I) and 17 were in accelerated phase or blastic crisis (group II). Patients were divided into 3 risk groups according to Hasford score: Low risk group: score ≤70, Intermediate risk group: score 71-1480 and High risk group: score >1480. After informed consent, analysis of phosphorylated STAT5 (pSTAT5) was done using Flow Cytometry. Results were expressed as percentage positivity and florescent ration in CD34 positive and CD34 negative cells. pSTAT5 expression was studied in relation to various hematopoietic and clinical parameters. Results: pSTAT5 was expressed in all cases tested. The level was statistically significantly higher in advanced phases than in the chronic phase (p<0.001). CD34 positive cells were pSTAT5 positive. CD34 negative cells were pSTAT5 negative (<10%) in 8/22 (36.4%) and 5/17 (29.4%) patients in group I and group II respectively. pSTAT5% expression was significantly higher in group II as compared to group I (56.4±27.6% vs. 55 ± 21%, respectively; p<0.001). All CD34 positive cells were pSTAT5 positive. pSTAT5 expression was significantly higher in Group II than in Group I but the difference did not achieve statistical significance. pSTAT5% expression showed significant positive correlation with both peripheral blood and bone marrow blast percentage (r=0.39 and 0.37; p=0.017 and 0.02 respectively). The distribution of the 3 Hasford risk groups among the two patients groups revealed 8/21 (38.1%) low risk, 2/21 (8.1%) intermediate risk and 5/21 (23.8%) high risk in Group I as compared to 4/16 (25%), 8/16 (50%) and 4/16 (25%) in group II; the difference is statistically insignificant. No correlation was encountered between pSTAT5 expression on one side and age, Hasford score or duration of chronic phase on the other side. Summary/Conclusions: The level of expression of pSTAT5 is higher in advanced phases of CML reflecting a higher tyrosine kinase activity of the BCR/ABL chimeric protein. It may serve as an indicator of the BCR/ABL expression level.

1203
TREATMENT WITH IMATINIB INHIBITS NF-KB AND AP-1 ACTIVATION AND INTRACELLULAR CALCIUM LEVELS INSP3 OR ATP-INDUCED IN CML PATIENTS
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After InsP3 and ATP administration (patients displayed decreased intracellular calcium [Ca2+]i fluxes both which leads to malignant transformation. Our previous results demonstrated that peripheral blood mononuclear cells derived from CML patients displayed decreased intracellular calcium ([Ca2+]i) fluxes both after InsP3 and ATP administration (Ciarcia et al. J.Cell.Physiol., 2010). On the other hand several transcription factors are activated in response to physiopathological increases and intracellular calcium levels. It is widely known that BCR-ABL can inhibit apoptosis also by activating at cytoplasmic level the FISK/AKT pathway and furthermore, the important anti-apoptotic gene expression are increased through transcription of NF-kB/Rel, appears activated in CML patients. Aims. In present study we evaluated the levels of transcription factors NF-kB and AP-1 as well as the intracellular calcium measurement in peripheral blood leukocytes of control or CML patients before and after treatment for three months with imatinib 400 mg in order to determine whether treatment with imatinib was able to change these parameters. Methods. NF-kB, AP-1 and intracellular calcium levels were measured on lymphocytes isolated from blood of n. 8 healthy volunteers and n. 8 CML patients in first diagnosis of CML and after three months treatment with imatinib. Intracellular Ca2+ concentrations were measured by using the radiometric fluorescent indicator dye FURA-2/AM, the membrane-permeant form of FURA-2/AM as previously described and opportually modified by Pagnini U et.al. (Anticancer research, 2000). To detect and quantify NF-kB and AP-1 activation in our samples, we used ELISA-based Trans-Am transcription factor kits (Active Motif, Carlsbad USA). Results. Our results showed that InsP3 induced an increase in maximum levels of [Ca2+]i by depletion of the intracellular calcium stores with a significant higher effect in CML untreated than treated patients for three months with imatinib increasing from 121.9±8.1 to 573.5±29.7 nM in CML untreated and from 120.3±7.4 to 252.1±18.7 nM (-55.1 %) in treated patients. ATP increased intracellular Calcium concentration by Ca2+ influx with a significant higher effect in CML untreated from 115.2±6.4 to 251.8±22.3 nM than treated patients from 121.4±7.6 to 197.3±14.2 nM (52.9 %). The results of NF-kB and AP-1 in CML samples, demonstrated that respect to the constitutive expression of NF-kB and AP-1 in primary CML lymphomonocytes, Imatinib treatment was able to significantly reduce NF-kB (-92.7%) and AP-1 (-84.6%) activation. Conclusions. Our results suggest that the inhibitory activity of imatinib on both intracellular calcium levels and NF and AP-1 activation could be used as a prognostic factors to address the follow-up in patients with CML treated with imatinib.

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HIGH RESOLUTION MELTING CURVE ANALYSIS FOR THE DETECTION OF SNPS IN CYP3A4 OF SESOTHO CML PATIENTS BEING TREATED WITH IMATINIB

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Background. Tyrosine kinase inhibitors (TKIs) have become the treatment of choice for chronic myeloid leukemia (CML). However, up to 5% of patients may experience intolerance to TKIs as a result of adverse drug reactions (ADRs). TKIs are primarily metabolized by Cytochrome P450 enzyme CYP3A4. Although it appears that the metabolism of TKIs, specifically imatinib, is not affected by the inhibition of CYP3A4, the role of this enzyme in ADRs to TKIs is uncertain. Allelic variants based on single nucleotide polymorphisms (SNPs) in CYP3A4 have been found to be associated with altered catalytic activity in exons 5, 7, 10, 11 and 12 that may potentially contribute to inter-individual differences in drug metabolism. Since SNP profiles differ between populations, it is important to screen all 13 exons of CYP3A4. Unfortunately, sequencing all the exons of CYP3A4 is time consuming, laborious and expensive. However, high resolution melting curve (HRM) analysis has successfully been used to screen for SNPs in different genes reducing the need for unnecessary sequencing. Aims. To determine whether HRM analysis can be used to screen for SNPs in CYP3A4 of Sesotho CML patients. Methods. Blood samples were obtained from 38 Sesotho CML patients being treated with imatinib after obtaining informed consent. DNA was extracted from samples after Trizol stabilization. HRM primers were designed for exons 5, 7, 10, 11 and 12 using the online primer design program Primer3Plus. HRM analysis was performed using MeltDoctor HRM reagent on the ABI 7500 Fast. Samples were sequenced using the BigDye Terminator v3.1 cycle sequencing kit. Results. Of the 38 patients screened by HRM analysis, only 3 were found not to have any SNPs. The range in Tm for variant samples overlapped with that of the reference sample making it difficult to distinguish between them based on the use of the Tm. In comparison, the use of difference plots identified a total of 3 variants in exon 5, 20 in exon 7, 25 in exon 10, 2 in exon 11 and 13 in exon 12 with SNPs. Of the 27 SNPs identified, 21 have not been previously described. Conclusions. HRM analysis was successfully used to detect the presence of single and multiple SNPs in exons 5, 7, 10, 11 and 12 of CYP3A4 prior to sequencing. A total of 21 SNPs, not previously described, were identified in Sesotho patients. The use of HRM analysis in this study allowed the exclusion of 112 exon sequencing reactions which is a major cost saving factor in SNP screening. The effect of these SNPs on CYP3A4 gene expression now needs to be tested.

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CYTOGENETIC CHANGES IN PHILADELPHIA CHROMOSOME NEGATIVE CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA DURING IMATINIB TREATMENT

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**Background.** Chronic myeloid leukemia (CML) is a complex disease with strong inter-individual susceptibility. Human genetic variation is based on the presence of polymorphisms along the DNA sequence. Polymorphisms located at the promoter or the coding region of the gene could change the level of the encoded protein or the protein activity, respectively. To avoid the damage caused by oxidative stress or diverse environmental hazards such as, xenobiotics, the cell has developed several anti-oxidant and detoxification mechanisms. Genetic alterations in components of these protection mechanisms have been associated with cancer susceptibility. Aims. The aim of this study was to determine the association among CML susceptibility and polymorphism located in a battery of anti-oxidant and detoxification factors.

**Methods.** - We collected blood sample from 250 individuals with CML and 250 healthy subjects. Cases and controls were matched by age, gender and ethnicity. Single nucleotide polymorphism (SNPs) at the genes GSTP (A1404G) and NQO1 (C609T) were genotyped by TaqMan assay, meanwhile short tandem repeat at HMOX1 promoter region (GTn) polymorphism was determined by Gene Scan analysis. Significant differences in allele and genotype frequency distribution and odd ratios (OR) was determined by Chi-squared and Fisher’s exact test. After genotyping, all polymorphisms tested were in Hardy-Weinberg equilibrium in both, case and control population.

**Results.** - We found no significant differences in allele and genotypic distribution of GSTP and NQO1 SNPs between CML and healthy individuals. 

**Conclusions.** - Using the K562 (t9;22) CML model, we examined the imatinib effect on the BCR-ABL fusion protein, Bcr-Abl fusion mRNA, cell viability, and cell differentiation. Methods. The K562 cells were continuously exposed to 5 μM of imatinib; imatinib was replenished at 24, 48, and 72 hours. Cells were harvested at 24, 48, and 72 hours and underwent protein, mRNA, viability, and differentiation analysis. BCR-ABL fusion protein in the cell lysate was quantitatively measured at each time-point using the BD® Cytometric Bead Array Research Use Only BCR-ABL Protein™ (BD Biosciences, San Jose, CA, USA). Bcr-Abl mRNA transcripts were measured using Q-PCR assay (Ipsogen Corp, Stamford, CT). Cell viability was determined by trypan blue staining and apoptosis was determined by flow cytometry through Annexin V/7-AAD double staining. Erythroid differentiation was scored by benzidine staining and Glycoporphin A as hemoglobin synthesis and erythroid lineage markers by flow cytometry, respectively. Exposure to imatinib for 24, 48, or 72 hours reduced the BCR-ABL protein concentration by 50%, 70%, and 90%, respectively (Figure 1). At the same series of time points, the Bcr-Abl mRNA transcript level was reduced by 20%, 70%, and 90%, respectively. The fusion protein showed significantly faster reduction than fusion mRNA at 24 hours. The trypan blue and Annexin V/7-AAD stainings showed that the continuous drug exposure induced cell apoptosis in a time-dependent manner.
tive leukemia such as chronic myeloid leukemia (CML) and Philadelphia chromosome (Ph) positive acute lymphoblastic leukemia (ALL), however, imatinib resistance is often reported in patients with advanced-stage disease by the mutations in kinase domain (KD) of BCR-ABL. Nilotinib is also a potent and selective inhibitor of BCR-ABL tyrosine kinase. In the phase 3 Evaluating Nilotinib Efficacy and Safety in Clinical Trials: Newly Diagnosed Patients (ENESTnd) clinical trial, nilotinib was demonstrated superior efficacy to imatinib with higher and faster molecular responses against CML patients. Aims. Nilotinib is the very high rates of responses were achieved during the first 12 months on treatment, however, there are fully not known about nilotinib resistance. Methods. We established the imatinib (K562 IM-R) and nilotinib (K562 AMN-R) resistant cell lines and treated the cell lines with the IC50. 4) Nilotinib proliferation assay, microarray assay and immunoblot assay. Results. We first evaluated the gene expression profiles and intracellular signaling of CML cell line K562 exposed to imatinib or nilotinib. When their gene expression profiles were compared, there was an increase of 994 genes in imatinib and 1298 genes in nilotinib and 854 genes overlapped. In contrast, there was a decrease of 582 genes in imatinib and 816 genes in nilotinib and 515 genes overlapped. In apoptosis related gene, Myb and Myc gene was decreased in nilotinib treatment. We next examined the drug sensitivity of imatinib and nilotinib resistant cell line. Cell proliferation of K562 IM-R and K562 AMN-R did not decrease after imatinib (10 μM) or nilotinib (2 μM) treatment compared with parental cell line, K562. Imatinib resistance occurs through a variety of mechanisms, including BCR-ABL kinase domain mutation, and amplification or over-expression of BCR-ABL. The BCR-ABL expression was not enhanced in these cell lines compared with K562 by FISH and immunoblot analysis. The BCR-ABL kinase domain mutation was not found in K562 IM-R and K562 AMN-R cells by direct sequence analysis. We next examined the intracellular signaling by using these cell lines. One of the Src family kinase, Lyn was activated in resistant cells. The protein expression of Lyn was also enhanced. Ponatinib (AP24534), an oral multiple tyrosine kinase inhibitor, is a potent pan-BCR-ABL inhibitor with activity against imatinib resistant cells. We next examined the efficacy of ponatinib against imatinib and nilotinib resistant cell lines. We found the cell proliferation was decreased after ponatinib treatment. We also found the phosphorylation of BCR-ABL, Lyn and Crk-L was reduced and FAK (ADP-ribosyl) polymerase (PAR) was activated after ponatinib treatment. Summary. These results suggest that the expression and protein activation signatures identified in this study provide insight into the mechanism of resistance to imatinib and nilotinib and we demonstrate ponatinib has anti-leukemia effect by reducing ABL and Lyn kinase activity. 1210 COMPARISON BETWEEN TWO QUANTITATIVE REAL TIME PCR FOR BCR-ABL DETECTION IN CML: EAC METHOD AND GENEEXPERT C López-Jorge,1 MT Gomez Casares,2 A Jiménez-Velasco,2 M García-Bello,3 S De la Iglesia,1 T Ramírez,1 M Alcala,1 J Lopez Brito,1 G Sanchez Sanchez,2 A Heiniger,2 T Molero1 1Hospital Universitario de Gran Canaria Doctor Negrín, Las Palmas de Gran Canaria, Spain 2Hospital Carlos Haya, Málaga, Spain 3CAE Vecindario, Las Palmas, Spain Background. Recently, some efforts have been made in Europe in order to standardize BCR-ABL quantitation as well as to certify laboratories which are able to express the results in an international scale applying a conversion factor (CF). However, light variations in the techniques which are used can cause CF modifications. The GeneXpert is an automated closed system for the quantification process, performing a nested-PCR using ABL as control gene. AIMS: To determine whether BCR-ABL results obtained by GeneXpert are comparable with the ones given by the standard method with the CF. Material and Methods. BCR-ABL was determined by both methods in 58 samples from bone marrow and/or peripheral blood of patients with CML (25 in Carlos Haya Hospital; 33 in Dr. Negrín Hospital). For the analysis with the standard method, LightCycler platform was used according to Europe Against Cancer (EAC) conditions. Both laboratories showed the results in an international scale, applying the GeneXpert CF. GeneXpert CF analysis was performed according to manufacturer instructions. With both methods, the quantification is automated by every equipment software and the final result is expressed as BCR-ABL/ABL x 100. The obtained results were statistically analyzed by Passing Bablok method in order to establish the relation between these two methods and Bland and Altman were employed to estimate the measurement error. RESULTS: GeneXpert results are slightly higher than the ones obtained by the standard method, being significant the difference of the means between the logarithm of the standard one correct-ed with CF and the logarithm of the GeneXpert (p<0.004). The concordance indicates that both techniques are significantly correlated, being the correlation coefficient of 0.9507 (Pearson correlation factor of 0.9571). Besides, it doesn’t exist a significant deviation of linearity (P=0.10) between both measurements. Finally, according to the standard technique and after applying the CF, 28 samples were in MMR (≥0,1%). Out of them, 22 matched with the results given by the CF. The other 6 showed levels of ≥0,1% and very close (range 0,11-0,23%). Just 2 samples in MMR by GeneXpert didn’t show the same result with the standard method once CF was applied (0,11% and 0,12%). Conclusions. 1) The concordance level between both measurements is quite good. 2) The result obtained with the standard method can be predicted by GeneXpert, although slightly overestimated. Both measurements showed a relevant difference between standard variable scores and GeneXpert, so that we can consider GeneXpert as an alternative and useful method to determine molecular response.
(CML) has shown interesting effects also on the patients immune cells and therefore on immune escape mechanisms. Anti-tumor immune reactions in cancer patients may be hampered by immune escape mechanisms, including recruitment of suppressor cells like myeloid-derived suppressor cells (MDSC) or expression of immune inhibitory molecules such as programmed death receptor ligand 1 (PDL1) on tumor cells and programmed death receptor 1 (PD1) on T cells. Immune inhibitory cell surface markers PD1 and PDL1 expression in patients with chronic myeloid leukemia (CML) has shown interesting effects also on the patients immune cells. As the majority of CML patients treated with Imatinib mesylate have not been extensively studied. Aims. The aim of the study was to investigate immune escape mechanisms in CML patients. Methods. Cytopreserved leukapheresis samples (n=18) from high (n=11) and low (n=7) Sokal score risk group CML patients and buffy coats from healthy controls (HC, n=21) were investigated for the presence of MDSCs (CD11b+CD14+CD33+) and the expression level of immune inhibitory surface markers PD1 and PDL1 by flow cytometry. The level of the MDSC-associated molecule Arginase-1 in cells from CML patients (n=5) and controls (n=5) was assessed by real time PCR. T cell proliferation upon tumor cell encounter was determined by flow cytometry. Results. The level of MDSCs in leukapheresis samples from CML patients was significantly higher compared to the levels in healthy controls (medians 1.2% in CML and 0.8% in HC). When CML patients were divided into Sokal high and low risk groups the high risk group had higher MDSC level (median 3.6%) compared to the low risk group (median 0.8%). The level of MDSC in Sokal low risk group did not significantly differ from the level in HCs. Arginase-1 is a molecule linked to immune escape mechanisms of tumor cells. The level of Arginase-1 was higher in CML patients compared to HCs as assessed by real time PCR. Further, the expression of PDL1 on CD11b+ myeloid cells was higher on cells from CML patients compared to controls. There was no difference in PD1 expression levels between Sokal low risk and Sokal high risk groups. PD1 is known to inhibit T cells by binding to PD1 cell surface receptor. In our study the expression level of PD1 on CML T cells was higher compared to the expression of PD1 on healthy T cells. However, blocking PD1 could not increase proliferation of healthy T cells mixed with CML cells. Conclusions. CML exert multiple immune escape mechanisms including high levels of MDSCs, Arginase-1, PD1 and PD1 expression. Interestingly, some mechanisms investigated seem to correlate with Sokal score demonstrating the relevance of immune biomarkers in CML. Treatment with TKIs lowers tumor burden and affect immune cells. It remains to investigate if TKIs could affect immune escape mechanisms in CML leading to long lasting anti-tumor control.

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THE B-ABL TRANSCRIPT LEVELS IN CML PATIENTS AT PRESENTATION HAVE NO PROGNOSTIC SIGNIFICANCE FOR THE MOLECULAR RESPONSE TO IMATINIB
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Background. The chronic myeloid leukemia (CML) outlook has radically changed since treatment with Imatinib mesylate. Responce prediction has proven useful and might guide therapeutic decisions in the era of targeted therapy. As the majority of CML patients treated with Imatinib achieve a complete cytogenetic remission (CCR), there is a need for a molecular analysis that measures the level of B-ABL transcripts in order to detect minimal residual disease. Aims. We have studied the significance of B-ABL transcript levels at presentation for the response to treatment with Imatinib. The B-ABL levels were measured during 18 months of treatment with Imatinib. Methods. We have monitored B-ABL transcript levels by RQ-PCR in 57 CML patients with CML-CP starting at presentation. The B-ABL transcript level at baseline was considered in order to evaluate a correlation with MMR achievement. Transcript quantification by RQ-PCR was measured every 3 months from baseline. As a national reference laboratory for CML molecular monitoring, a conversion factor (CF=0.7838) was used for International Scale alignment. Results. 85% of the patients that achieved CCR by 6 months have a baseline transcript level range between 4-78%. 15% of patients achieved major molecular response (MMR) at 6 months, having baseline transcript levels between 21-61%. The remaining 32% of patients achieved MMR at 12 months and transcript level at baseline was between 4-75%. The type of transcript was about 60% b3a2 and 40% b2a2 in these three groups. Conclusions. Achieving an early MMR seems to show no correlation with B-ABL transcript levels at diagnosis. The level of B-ABL transcripts at presentation should not be a prognostic factor for the response to Imatinib treatment. This study is in progress, in order to extend the group of patients.

1214
SLC22A1 POLYMORPHISMS ARE NOT ASSOCIATED WITH CYTOTOGENIC AND MOLECULAR RESPONSE TO IMATINIB MESYLATE IN CHRONIC MYELOID LEUKEMIA PATIENTS
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Background. The roles of drug efflux and drug influx proteins have been investigated in the development of resistance to imatinib mesylate (IM). The activity of IM has recently been linked with the organic cation transporter 1 (OCT1), an influx transporter, which is codified by SLC22A1 gene. However, few studies have shown the effects of SLC22A1 polymorphisms in cytogenetic and molecular response to IM in chronic myeloid leukemia (CML) patients. Aims. To investigate the relationship between SLC22A1 polymorphisms and response to IM in patients with chronic myeloid leukemia (CML). Methods. One hundred and eighteen CML patients in the chronic phase of CML, both genders with an age range of 18 to 80 were studied. All patients were initially treated with a standard dose of IM (400 mg/day) and divided into two groups according to their response. The first group (responder) comprised 70 patients who had a complete cytogenetic response within 18 months of treatment. The second group (non responder) comprised 48 patients who did not have a complete cytogenetic response with the initial dose (400 mg/day) of IM or who relapsed during treatment and were submitted to higher doses of 600 or 800 mg/day. Criteria of failed response to treatment were based on the European Leukemia Net. Patients with cytogenetic patterns other than the Philadelphia chromosome and/or with mutations in the BCR-ABL1 gene were excluded from this study. Major molecular response (MMR) was defined as a reduction of BCR-ABL1 transcript levels to ≤ 0.1% in the peripheral blood standardized on the international scale. The SLC22A1 (848C>T (rs4646277), 859G>C (rs4646278), 1222A>G (rs628051) and 480G>C (rs583569) polymorphisms were detected by Real Time PCR. Results. Minor allele frequencies for SLC22A1 polymorphisms were similar between responders (859G: 8.7%; 1222G: 74.6% and 480G: 12.3%) and non responders (859G: 6.2%; 1222G: 67.2% and 480G: 12.5%; P=0.05). The 848C>T variant was not detected in this sample. The frequencies of SLC22A1 (859G>C, 1222A>G and 480G>C) haplotypes in both groups were also similar (P=0.05). Association between SLC22A1 genotypes or haplotypes and MMR was not found in CML patients independently of type of IM response. Conclusions. SLC22A1 859G>C, 1222A>G and 480G>C polymorphisms do not influence the cytogenetic and molecular response to IM in CML patients.

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1215
VARIANT PHILADELPHIA TRANSLOCATIONS - CYTOTOGENIC EVOLUTION IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA
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Background. The Philadelphia chromosome (Ph) is the hallmark of chronic myeloid leukemia (CML). Whereas the majority of Ph-positive CML patients show the standard Ph translocation involving chromosomes 9 and 22, t(9;22)(q34;q11), the minority of cases presents a variant type of Ph translocation. Available data indicate that variant Ph translocations do not confer any specific phenotypic or prognostic impact as compared to CML with a standard Ph chromosome. Methods. The study was conducted between January 2002 and January 2011. The cytogenetic studies were made on hematopoietic bone marrow using culture for 24-48 hours on a culture medium dedicated for hematopoietic cells, followed by standard cytogenetic exam, GtG banding and karyotyping. The hematologic and cytogenetic evaluations of patients were monitored during the treatment. Results. Out of 87 patients with chronic myeloid leukemia cytogenetic analyzed, 8 patients had Philadelphia (Ph) negative, 71 patients had Ph positive, of which 5 patients carried a variant Ph translocation. Four patients had complex translocations involving 3 chromosomes: t(6;9;11), t(5;6;11), t(6;9;22), t(6;9;22)(p21.3;q34;q11), t(6;9;22)(p21.3;q34;q11), t(7;9;22)(p22;q23;q11) and (9;11;22)(p23;q21.3;q11). One patient had a complex variant Philadelphia (Ph) translocations, t(8;19;22), with no obvious involvement of chromosome...
PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA (CML) ACCORDING TO CLINICAL AND EPIDEMIOLOGICAL DATA OF REGIONAL MONITORING

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OBJECTIVES. The present findings strongly suggest that variant Ph translocations of CML occur as primary cytogenetic changes similar to the classical Ph1 translocations. Some complex chromosomal rearrangements are associated with rather poor prognosis and respond poorly to antileukemic treatment.

1216 THE EFFECT OF IMATINIB TREATMENT OF CHRONIC MYELOID LEUKEMIA (CML) ACCORDING TO CLINICAL AND EPIDEMIOLOGICAL DATA OF REGIONAL MONITORING

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BACKGROUND. Since 2005-2007 Imatinib (IM) has been the current standard of care for chronic myeloid leukemia patients with CML in the Russian Federation and is financed by the federal program. Aims. To evaluate the clinical effectiveness of IM in unselected population in the real-life out-patient therapy and epidemiological rates of CML on the basis of the regional monitoring from 2000 to 2010. Methods. We analyzed the data of the regional register of CML-patients. Clinical effectiveness criteria: frequency of no failure after 18 months according to the ELN 2009, cumulative frequency of complete cytogenetic response (CCgR) at the moment of the last research after ±12 months of IM treatment. The analysis of IM effectiveness has been carried out in the three groups of patients: 1st - the early chronic phase (ECP) - ±6 months from the establishment of CML till the beginning of IM treatment, 2nd - the late chronic phase (LCP) - ±6 months from the establishment of CML till the beginning of IM treatment (7 to 127, median 23) and 3rd - the progression phase (PrP) which combined the patients with the accelerated phase and the blast crisis. Epidemiological rates: incidence, prevalence, mortality were calculated by the standard methods of the variation statistics and trends. Statistical validity was determined by the method Chi-square. Results. Since 2000 CML Ph-positive was established in 248 patients, the age (13-82 years, a median 52, including 2 children cases), sex (M-112, F-135), 161 CML patient have received IM treatment: 76 in ECP, 75 in LCP and 10 PrP. The frequency of no failure after 18 months and the cumulative frequency of CCgR at the moment of the last research after ±12 months of IM treatment are presented in the table 1. Nobody in PrP has not reached CCgR. The IM treatment has been stopped in 42 (25,5%) to 161 patients. The average multyear mortality rate was 0,77±e0,08/0000: max 1,040/0000 in 2004, min 0,640/0000 in 2008, without trend (T=0,04%). The quantity of deaths of CML patients was 197. The average multyear mortality rate was 0,6±0,120/0000: max 1,1/0000 in 2000, min 0,170/0000 (in 2010), - with a marked trend to decreasing for 10 years of mortality (T=-6,6%). The average multyear prevalence rate was 5,6±0,420/0000: max 9,4/0000 in 2010, min 2,7/0000 in 2002, - with a marked trend to growth of CML prevalence for 10 years (T=+7,8%), especially for the last 5 years. Conclusions. IM in real-life outpatient therapy of CML patients has shown high clinical effectiveness without changes of average multyear incidence rate with decreasing average multyear mortality rate and increasing average multyear prevalence rate.

1217 LONG-TERM RESULTS OF IMATINIB THERAPY AND SURVIVAL IN PATIENTS WITH CHRONIC PHASE CHRONIC MYELOID LEUKEMIA RESISTANT OR INTEROLANT TO INTERFERON- ALPHA THERAPY

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BACKGROUND. In Russian Federation a selective BCR/ABL tyrosine kinase inhibitor (TKI) imatinib (IM) was introduced for the first time in 2000y for chronic phase (CP) CML patients(pts) after interferon α (IFN-α) failure. The 2nd and more potent generation of TKI (TKI-2) became available since 2005-2007y. At present time we summarize the long-term therapy results.

OBJECTIVES. To evaluate the efficacy of 1st and 2nd generation TKI therapy: frequency and stability of complete cytogenetic response (CCyR), frequency of major molecular response(MMR) and complete molecular response (CMR), incidence of second tumors and overall survival (OS). PATIENTS AND TREATMENT. The analysis included 79pts. Criteria for inclusion into an open non-randomized study were: CP CML, diagnosis confirmation by standard cytogenetic study; IFN-α therapy discontinuation and initiation of IM therapy from Jul-2000 till Sep-2001. Median (Me) age 39 (15-64) years, males: females =41:38. Distribution by Sokal groups: low-50 (63%) pts, intermediate + high - 15(19%) pts plus 14(16%) pts. Me duration of CML before IM therapy was 35,1 (8-157) months: less than 1 year in 12pts (15%), from 1 to 5 years in 50pts (63,5%) and more than 5 years in 17pts (21,5%). Mean period of IFN-α pretreatment was 26 (0,5-156) months. Me duration of IM therapy (Jul2010) was 80 (2,4-118) months. The initial dose of IM was 400mg/day. Me duration of TKI-2 therapy was 31 (2,5-51) months. 4(5%) pts received more than two TKIs. Me duration of CML (from diagnosis to July 2010) was 120,5 (18-259) months. Statistical analysis was performed using the package v.SAS9.1.3. Results. 59 of 79pts (75%) alive on Jul2010 received TKI therapy: IM 34 (43%) pts /TKI-2 24 (30%) pts/ 1(1,3%) pts treated by hydroxyurea (resistant to all available TKI). For the entire observation period CCyR was obtained in 64% (51pts). The cumulative incidence of CMR was 54% (45pts) with a Me to CCyR 9 months (5-53). Acquired cytogenetic resistance was observed in 16 of 43 (37%) pts (or 20% of the total group). CCyR was again achieved on IM for 27 (64%) pts (still on IM) and for 8(10%) pts on TKI-2 therapy for the first time. 34(43%) pts with CCyR continue IM therapy (27 of them have stable CCyR). MMR in pts with CCyR on IM therapy was observed for 21 (26,7%) of 79 pts, CMR was achieved in 11 (14%) pts.

1218 CYTOKINE SYNTHESSES BY T-CELL SUBSETS OF CML PATIENTS PRIOR TO AND DURING IMATINIB THERAPY: RELATION BETWEEN THE LEVEL OF RESPONSE TO THERAPY (OPTIMAL VERSUS NO OPTIMA) AND ENDogenous T-CELL FUNCTION

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OBJECTIVES. The majority of imatinib (IM) treated chronic phase chronic myeloid leukemia patients achieved complete haematological and cytogenetic remission but relatively few of them achieve complete molecular remission. Although T-cells cytokines role in the long term

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control of CML is well established, previous studies showed contradicting results regarding imatinib effect on the endogenous T-cell function by IM. The variability in the patients population included in these studies may contributed to this where in some of them cells were obtained from patients in CCR or patients with resistant/intolerance to previous therapy with IFN-alpha. Aims: the purpose of this study was to determine the relation between the endogenous T-cell function prior to therapy and response to IM therapy in CP CML. In addition, modulation of the endogenous T-cell function during IM therapy was studied. Methods: Using flow cytometry, we studied Th1 and Th2 cytokine synthesis by PMA activated CD4 and CD8 T cell subsets of 22 patients with newly diagnosed CML in chronic phase prior to and during IM therapy compared to that of 5 patients with IM resistance and 10 healthy donors. The percentage of Th1 and Th2 were compared to IFN-α producing levels. Results: In patients with optimal response, pre treatment cytokine studies showed lower Th1, but higher percentage of Th2 and TNF-α producing CD4 and CD8 T cells compared to that of healthy donors. Significant higher levels of Th2 and TNF-α producing CD4 and CD8 T cells were found compared to those from patient with no optimal response and those known to have IM resistance. Lower level of Th2 and TNF-α producing T cells were found in the last 2 named groups of patients compared to that of healthy donors. Cytokine responses of Th2 profile to IFN-α was detected early during IM therapy (6 weeks) in patients with optimal response coinciding with the CHR in addition to decline of TNF-α producing CD4 and CD8 T cells. This immunological response of T cell subset during IM therapy preceded both the cytogenic and molecular response which was maintained throughout the follow up period. In patients with molecular response, a significant increase in the percentage of IFN-α producing CD4 and TNF-α synthesizing CD4 and CD8 T cells at 6 weeks of therapy to levels exceeding that of healthy donors and those with optimal response followed by a rapid decline in the cytokine levels at later follow ups. Conclusions: CP CML patient with optimal response has Th2 cytokine and suppressed Th1 cytokine profile at diagnosis with restoration to normal levels during therapy. On the other hand patients with no optimal response and those known to have IM resistance showed suppression of Th1, Th2 cytokines and TNF-α. The role of pre treatment indigence IL-4 and TNF-α in determining the response to IM therapy needs further investigation.
goides-like reaction). Rare changes are hyperpigmentation, psoriasis and pityriasisiform eruptions. Aims: The aim of our study was to analyze cutaneous changes during IM treatment in large cohort of our CML pts. Methods: Between 2002 and 2010, 110 pts with CML were treated with IM in our institution. IM therapy was commenced in a dose of 400 and in some cases escalated to 800 mg/d.

Results. In our group, 76 patients (69%), 40 males and 36 females, mean age 39 years (28-78 yrs) had some skin changes. All pts were in chronic phase CML, 30 pts had low risk, 27 pts intermediate and 19 pts high risk Sokal score. Periorbital edema was the most common, occurring in 70 pts (64%), almost all had typical, mild form, pronounced in the morning. But 5 pts had also severe edema (CTC Gr. 2), without cessation of imatinib therapy. Edema developed in our group from two weeks to 20 months of IM treatment (median 12 months) and was treated by various means, e.g. low-salt diet, oral diuretics and in some cases topical 1% hydrocortisone. Six patients (5.4%) had other different cutaneous reactions. Two patients had acute maculopapular rash (CTC Gr. 2) with pruritus, needed topical steroids bid during two weeks. One patient had worsening of vitiligo with extension in areas and pronounced edge hyperpigmentation, and second one worsening of psoriasis but responded to more frequent psoriatic treatment. Two previously healthy patients had a severe, recidivante skin changes IM (CTC Gr. 2), responding to pause in IM treatment and to topical/systemic steroids. Unfortunately, in one patient due to recidivante skin changes IM treatment was discontinued for good. We have not found any correlation of any changes with Sokal score or some other pretreatment variable. Conclusions. Imatinib has many mild adverse events but the incidence and management of cutaneous side effects has been rarely reported. Most cutaneous eruptions caused by IM do not need discontinuation of treatment and are usually self limited. Administration of oral or topical corticosteroids can ameliorate some of imatinib induced cutaneous changes without need for treatment cessation.

ECONOMIC BURDEN OF PROGRESSION IN PATIENTS WITH PHILADELPHIA CHROMOSOME-POSITIVE CHRONIC MYELOID LEUKEMIA (PH+ CML) IN CHRONIC PHASE

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Background. The ENESTnd study showed that after 12 month of therapy, newly-diagnosed chronic phase (CP) Ph+ CML patients (pts) receiving nilotinib (600 mg BID) had a significant superior major molecular and complete cytogenetic response and improvement in the time to progression to accelerated phase (AP) or blast crisis (BC), compared with those receiving imatinib (400 mg QD) (P=0.01). Aims. To estimate the economic burden associated with early progression to AP in the US. Methods: A literature-based Markov model was developed to estimate the 5-year economic burden associated with early progression to AP despite first-line therapy. The model follows longitudinally cohorts of hypothetical pts who have progressed to AP after failure of first-line therapy in CP. Pts in the AP phase were assumed to initially receive therapy with a second generation TKI. These pts could discontinue this second generation TKI therapy due to intolerance or progression to BC. Patients who were off TKI (in the AP or BC) phase were assumed to receive primarily supportive care and/or transplant. The rate of transplantation in the latter patients was approximately 5% per year.

Prognosis was modeled using published data. Non-TKI-drug costs and productivity loss were assumed to increase as disease progressed. Quality of life varied by disease stage and treatment response. Resource use and costs were obtained from published estimates. Productivity was estimated using the human capital approach and considered the opportunity costs of CML-AP (i.e., productivity of progression minus productivity of non-CML age/gender-matched individuals). Results: Pts who have progressed to AP experience high direct medical ($314,294) and indirect costs ($176,179) (Table) and poor prognosis (5-year overall survival=24%), average survival 3.74 years of which 2.66 years in AP and 1.09 years in BC, average quality adjusted life years = 2.11). 87% of costs were assumed to occur in AP and 13% in BC. Overall, the total direct and indirect costs of progression were $490,473/pt. Conclusions. In addition to poor survival prognosis, CML disease progression is associated with a heavy economic burden. Preventing progression is therefore an important therapy goal in CML-CP.
diagnosis. The median follow-up on imatinib therapy is 44.8 months [2.53-122.24]. One hundred and thirty patients (80.7%) achieved a CCR as defined in the European LeukemiaNet criteria. The median follow-up in treatment with dasatinib is 35.3 months. The median time to MMR was 10.82 months [2.76-50.66]. The characteristics of those patients are detailed above (Table). The median duration of CMR was 15.65 months [0.00-51.31]. Conclusions. Preliminary results suggest that patients who achieved a CMR had a better MMR than other patients. The identification of factors correlated with CMR in CML-CP treated with imatinib as frontline therapy is ongoing.

Table. Characteristics of the 46 patients who achieved a CMR as defined previously.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=46</td>
<td></td>
</tr>
<tr>
<td>Male (%)</td>
<td>57%</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Median 56.3</td>
</tr>
<tr>
<td>Sokal risk score:</td>
<td>low 37, intermediate 43, high 15, unknown 5</td>
</tr>
<tr>
<td>Clonal evolution (%)</td>
<td>15</td>
</tr>
<tr>
<td>Imatinib initial daily dose</td>
<td>400 mg, 600 mg, 800 mg</td>
</tr>
<tr>
<td>Imatinib median time to CCR (months)</td>
<td>5.97 [2.62-50.66]</td>
</tr>
<tr>
<td>Imatinib median time to MMR (months)</td>
<td>10.82 [2.76-50.66]</td>
</tr>
<tr>
<td>Imatinib plasma level (%)</td>
<td>&lt; 1000 ng/ml, &gt; 1000 ng/ml, Unknown</td>
</tr>
</tbody>
</table>

**1224**

**DASATINIB EVEN AT LOWER DOSES IS AN EFFECTIVE FOR CHRONIC MYELOID LEUKAEMIA TREATMENT IN PATIENTS RESISTANT OR INTOLERANT TO IMATINIB**

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**Background.** Dasatinib is a multitargeted tyrosine kinase inhibitor (TKI) that is effective for the treatment of chronic myeloid leukemia (CML) patients resistant or intolerant to imatinib. Dasatinib recommended dose is 100 mg/day for patients in the chronic phase (CP) and 140 mg/day in the accelerated or blastic phases (AP, BP). However, it is not always possible to keep the dose. The major reason is CTCAE v3 grade 3/4 adverse events as well as persisting symptoms of prior treatment toxicity. **Aims.** To evaluate the efficacy of reduced dose of dasatinib on CML patients resistant or intolerant to imatinib managed in the context of every day clinical practice. **Methods.** We evaluated the outcome of 24/49 patients (48%) treated with reduced doses of dasatinib after the switch due to resistance and intolerance to imatinib. Group A: Daily dose of dasatinib was reduced in 14/24 patients due to haematological or non-haematological adverse events (AEs) grade 3/4 on standard dose. Group B: patients with persisting symptoms of AE, prior imatinib toxicity (10/24). Two regimens were used: dasatinib 50 mg/day or alternative doses of dasatinib 50 mg and 100 mg/day. The median duration of reduced dosing treatment was 6 months (3-24) in group A, with total dasatinib duration 40 months (10-60), and 10 months (2-15) from total 25 months (14-59) in group B. Patients were followed by routine haematological and cytogenetic assessment and molecular monitoring. Resistance patients (n=15) were screened for baseline BCR-ABL mutations, in 6 patients mutations were identified. **Results.** Group A: After dasatinib dose reduction there was no worsening in response quality (i.e. cytogenetic and molecular in 9/11 patients and quality of life improved with AE remission. The 10/11 (90%) patients survived for median 40 months (10-60), 9/11 (82%) without progression. In 5 patients 5 types of baseline mutations in BCR-ABL kinase domain were identified. Three of these completely disappeared during the treatment, in one patient the mutated clone declined from 100% to 25%. In one patient the mutated clone remained 100%. In two patients (with existing E255V and V299M mutation) the further progression to AP was solved with nilotinib followed in one with allotransplantation, while the second one died from progression. One patient interrupted dasatinib treatment for pregnancy period (10 months). After dasatinib readministration the re-achieved CCgR and MMR. Group B: CCgR was achieved in 8/10 patients (80%) and MMR in 5 of them after 4-12 months. In other 3 pts even CMR was accomplished. In 2 pts CCgR has not been achieved yet, one of them had baseline E459K mutation, which disappeared after 9 months. No other mutation occurrence was detected. All patients are alive with satisfactory quality of life with no progression. **Conclusions.** Our findings from clinical practice show the efficacy of reduced doses of dasatinib inducing or maintaining response in CML patients resistant or intolerant to imatinib. Further experience are warranted to confirm our findings.

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**1225**

**THERAPEUTIC RESULTS WITH IMATINIB IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA ACCORDING TO THE EUROPEAN LEUKEMIANET CRITERIA**

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**Background.** Imatinib has changed the evolution of CML from a disease with an average survival of 3-5 years to a disease with a survival of 86% at 7 years. The implementation of the ELN criteria allows us to check the minimal response and to change the therapy in case of sub-optimal response or therapeutic failure. The aims of this study are to evaluate the quality of the hematological, cytogenetic and molecular response, the overall survival and event free survival for 2 lots of patients treated with imatinib as a first and second line of therapy. **Methods.** This is an observational study, retrospective (01.01.2007-31.12.2010). 170 patients with CML in chronic phase have been treated and monitored in 2 departments of haematology from Romania (Fundeni Clinical Institute and Emergency Clinical County Hospital Brasov). The collected data have been analyzed using the SPSS programs (Kaplan-Meier log-rank test and Mantel Cox). Results. 56 patients (33%) treated with imatinib as a first line (median age of 41.5 years) and 114 patients (67%) treated with imatinib as a second line of therapy (median age of 50.4 years) have been included in this study. After 3 months of therapy, CHR was 91.07% in the first line lot and 84.2% in the second line lot. After 6 months, the rate of CHR was similar in both lots: 98.2% for the first line and 95.8% for the second line, without significant differences (p=0.508). The probability to lose the CHR until the 48th month is significantly higher for patients in the second line group comparing to the patients in the first line group (p=0.045). After 12 months, CCR was observed at 48.2% of first line patients and only at 17.5% of second line patients. The rate of MCR is 50% in the first line of therapy and 91% in the second line lot. The overal survival at 48 months is superior in the first line group (80.3%) compared to 52.6% in the second line group (p=0.0152). The overall survival at 48 months is similar in both lots: 94.6% in the first line and 88.6% for the second line (p=0.46).

**Conclusions.** Imatinib 400 mg/day induces high rates of CHR and MCR in CML patients treated with imatinib as a first line, without significant differences (p=0.00019). After 18 months, 33.9% of first line therapy patients achieved MMR, this type of response being sustained by 15% in the second line group. Event free survival for 48 months is superior in the first line group (80.3%) compared to 52.6% in the second line group (p=0.0152). The overall survival at 48 months is similar in both lots: 94.6% in the first line and 88.6% for the second line (p=0.46).

**1226**

**STUDY OF EFFECTIVENESS RECOMBINANT HUMAN ERYTHROPOIETIN IN CHRONIC MYELOID LEUKEMIA PATIENTS WITH ANEMIA INDUCED IMATINIB THERAPY**

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**Background.** Imatinib is a tyrosine kinases inhibitor (TKI) that revolutionarily changed prognosis and survival in patients with chronic myeloid leukemia (CML). It allowed to achieve not only cytogenetic but also molecular response. TKI may cause hematological toxicity according to anemia, leukocytopenia and thrombocytopenia. Aims. To investigate the effectiveness of Recombinant Human Erythropoietin (rHuEPo) in CML patients who developed anemia as toxic effect of Imatinib on erythropoiesis. Methods. 90 patients with CML received Imatinib in...
TKI therapy has been recognized in CML pts, but the frequency and metaphases during second-generation tyrosine-kinase inhibitors (2nd-TKI). After 48 mos, he developed grade II cytopenia and monocytosis (BCR-ABL mRNA transcript: 0.5%-0.9% according to International Scale, IS). Conventional karyotype was 46,XY, t(9;22)(q34;q11)/8, XY, -7[11]/46,XY, del(7)(q31,q35)[1]. Interphase FISH showed that monosomy 7 was present in 199/300 (66%) nuclei and BCR/ABL rearrangement in 18% nuclei. Conclusions. Literature data suggest that monosomy 7 is an ominous sign of disease progression in CML pts. The patient achieved a partial response and a near-major molecular response during treatment with a 2nd-TKI. This case is noteworthy, as it represents a therapeutic dilemma: the patient refuses both allotransplant from an unrelated donor and conventional chemotherapy, as without any treatment he feels well, with normal Hb level, and neutrophil and platelet counts. Under dasatinib, therapy with another TKI has no sense and new drugs effective in leukemic progression of Ph-negative myeloproliferative neoplasms have limited efficacy in Ph-positive CML.

1227 THE GROWTH OF A PH-NEGATIVE, MONOSOMY 7 CLONE ARSEN UNDER SECOND-GENERATION TYROSINE-KINASE INHIBITOR THERAPY IS UNEXPECTEDLY SLOW DOWN BY BCR-ABL IN A CHRONIC MYELOID LEUKEMIA (CML) PATIENT

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Background. The appearance of clonal abnormalities in the Ph-negative metaphases during second-generation tyrosine-kinase inhibitors (2nd-TKI) therapy has been recognized in CML pts, but the frequency and clinical significance of this event have not been assessed. Aims and Methods. The present study describes the unusually indolent course of a Ph-positive CML patient in chronic phase treated with nilotinib and dasatinib. Despite high efficiency of modern treatment of CML, the issue of disease resistance remains important. Among its main contributors are BCR/ABL gene mutations; however, their significance for occurrence of resistant clone is a subject of debate. This study comprises results of mutation analysis in a small group of patients with different stages of CML, including newly diagnosed. BCR/ABL mutation status was investigated in 15 patients tested for mutations at treatment change to nilotinib; 10 patients included 2 chronic phase subjects and one in blast phase (BP). The group of newly diagnosed patients included 2 chronic phase subjects and one in blast phase (BP). None of them was found positive for mutations. The second group included 15 patients tested for mutations at treatment change to nilotinib, the only 2nd line TKI registered in Ukraine. One patient was investigated due to decision to switch from interferon to imatinib. BCR/ABL mutations were detected in 6 of these patients (37.5%). In one case M511T mutation was detected. Treatment with nilotinib was started and appeared ineffective, despite reportedly high sensitivity of this mutation to nilotinib. Another patient lost CHR after 18 months of nilotinib. An other patient did not achieve complete cytogenetic response after 12 months; major molecular response and no BCR/ABL mutation at 18 months. The second patient with this mutation at 6 months achieved no response at all. The last patient of this subgroup with Y245H mutation was also resistant to nilotinib. In the second subgroup of 10 pretreated patients with no BCR/ABL mutations three responded to their treatment including one achieving complete molecular response. One patient lost CHR after 18 months of nilotinib. Another subject continued in CHR, yet no CyR was found at 6 months. The remaining patients in this group maintain their CHR; follow-up assessments of CyR were not yet performed. Conclusions: the data presented emphasize the controversy in the influcence of BCR/ABL mutations on the efficacy of CML therapy taking into consideration different results of nilotinib treatment in 2 patients with F359V mutation; unsatisfactory treatment results of patient with M511T, considered as sensitive to TKIs; lack of treatment response in some of the patients without BCR/ABL mutations. Undoubtedly, T315I mutation is an indication for allogeneic transplantation, but significance of other kinase domain mutations for CML treatment selection needs further clarification.
MEAN DAILY DOSE, MEASURED FROM 2–6 MONTHS OF IMATINIB THERAPY AS AN INAPPROPRIATE PREDICTOR FOR CYTOGENETIC AND MOLECULAR RESPONSE IN MONTH 6

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Background. Imatinib, tyrosine kinase inhibitor for BCR-ABL of CML has shown favorable results in clinic. Aims. This study was designed to observe the relationship among the response rate of imatinib (CyR, cyogenetic response, MR, molecular response), imatinib mean daily dose of month 1 treated, mean daily dose from month 2–6, imatinib plasma trough blood level of day 29 and day 6 and Sokal score. Methods. 21 patients were received imatinib 400mg QD as initial treatment after diagnosed as CP-CML were involved and cytogentic and molecular assay are performed at baseline and every 3 month. Within 22-26 h after the previous dose of imatinib, blood sample were collected for measurement of imatinib plasma trough blood levels at day 29 and month 6. Results. When we compared the group achieving the complete CyR (CCyR) (n=14/21) and group not achieving CCyR (n=7/21), it has significant difference (p=0.002) in their mean daily dose from month 2–6 (means; 389.64 ± 25.1 and 362.86 ± 46.5, respectively). However, there is no significance in mean daily dose from month 1, imatinib plasma trough blood level of day 29 and day 6 and Sokal score between these two groups. Group achieving 2-log reduction in MR in month 6 (n=14/21) has significant difference (p=0.000) in their mean daily dose from month 2–6 (395.42 ± 14.7 and 351.28 ± 45.7, respectively) compared with group not achieving 2-log reduction (n=7/21). Regarding CCyR, there is no significance in mean daily dose from month 1, imatinib plasma trough blood level of month 1 and 6 and Sokal score between these two groups. To observe the correlation with mean daily dose from month 1 and month 2–6, CyR and MR of month 6 and Sokal score, imatinib plasma trough level of month 1 and 6 respectively were grouped into 4 quartile. On the analysis based on quartile, imatinib plasma trough level of month 6 showed correlation with that of month 1 (p=0.024) and imatinib plasma trough level of month 1 had weak correlation with MR of month 6 (p=0.052). Conclusions. From statistical analysis, mean daily dose from month 2–6 showed correlation with CCyR and MR achieving 2-log reduction from baseline in month 6. This study on the process may show more relationship of the response rate of imatinib, imatinib mean daily dose of month 1 treated, mean daily dose from month 2–6, CyR and MR of month 6 and Sokal score in the further follow-up.

THE IMPACT OF THERAPY INTENSIFICATION AND OUTCOME OF 6 CML PATIENTS WITH THE SAME TYPE OF BCR-ABL MUTATION M244V

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Mutation in the kinase domain of BCR-ABL is not always the direct cause of imatinib resistance in CML patients. Also degree of mutation resistance to imatinib is very variable in different patients. The aim of this study was to evaluate the outcome and resistance overcoming by therapy intensification of 6 CML patients who on imatinib developed the complete CyR (CCyR) (14/21) and group not achieving CCyR (7/21), it has significant difference (p=0.002) in their mean daily dose from month 2–6 (means; 389.64 ± 25.1 and 362.86 ± 46.5, respectively). However, there is no significance in mean daily dose from month 1, imatinib plasma trough blood level of day 29 and day 6 and Sokal score between these two groups. Group achieving 2-log reduction in MR in month 6 (14/21) has significant difference (p=0.000) in their mean daily dose from month 2–6 (395.42 ± 14.7 and 351.28 ± 45.7, respectively) compared with group not achieving 2-log reduction (7/21). Regarding CCyR, there is no significance in mean daily dose from month 1, imatinib plasma trough blood level of month 1 and 6 and Sokal score between these two groups. To observe the correlation with mean daily dose from month 1 and month 2–6, CyR and MR of month 6 and Sokal score, imatinib plasma trough level of month 1 and 6 respectively were grouped into 4 quartile. On the analysis based on quartile, imatinib plasma trough level of month 6 showed correlation with that of month 1 (p=0.024) and imatinib plasma trough level of month 1 had weak correlation with MR of month 6 (p=0.052). Conclusions. From statistical analysis, mean daily dose from month 2–6 showed correlation with CCyR and MR achieving 2-log reduction from baseline in month 6. This study on the process may show more relationship of the response rate of imatinib, imatinib mean daily dose of month 1 treated, mean daily dose from month 2–6, CyR and MR of month 6 and Sokal score in the further follow-up.
treatment. CHR was preserved in 129 (84.5%) patients. Cytogenetic monitoring was performed in 148 patients. At present: 90 patients (60.8%) had CCgR, 17 (11.5%) PCgR, 2 (1.4%) minor cytogenetic response, 10 (6.8%) minimal cytogenetic response and 29 (22.3%) had no cytogenetic response. Molecular response was assessed in 106 cases: 40 (37.7%) patients are in complete molecular response, 20 (18.9%) - major molecular response, 46 (43.4%) patients had no molecular response. Conclusions. Effectiveness of TKI in routine practice is similar to results obtained in most of clinical trials. Strict cytogenetic and molecular monitoring of minimal residual disease is of great value to accurate response assessment and timely switch to second generation of TKI.

### Table 1. Daily mean dose and mutation frequency.

<table>
<thead>
<tr>
<th>Daily mean dose</th>
<th>Mutation frequency</th>
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<tr>
<td>293 mg/day</td>
<td>47 (34.3%)</td>
</tr>
<tr>
<td>315-349 mg/day</td>
<td>46 (34.3%)</td>
</tr>
<tr>
<td>350-399 mg/day</td>
<td>20 (15.2%)</td>
</tr>
<tr>
<td>≥400 mg/day</td>
<td>10 (7.6%)</td>
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### Results.

Fourteen out of 83 CML patients (17%) showed cytogenetic resistance to first-line IM treatment, and T315I mutation could be detected in two of them (14%). Patient 1 failed to reach cytogenetic response after 6 months of treatment, and was also resistant to dasatinib and nilotinib. He received a reduced intensity allogeneic transplantation, with autologous recovery and maintenance of the T315I mutation at three months of follow-up post-transplant. Patient 2 lost cytogenetic response after 12 months of treatment with IM. He received a myeloablative syngeneic transplantation and remained in complete molecular response eight months after transplantation. T315I levels at diagnosis, and after 3, 6, 9, 12 and 15 months of treatment with ITKs are shown in table 1. Conclusions. In our series, the incidence of T315I mutation in chronic phase CML patients with cytogenetic resistance to IM was similar to that reported in previous publications. The quantification of mutated copies along the patient follow-up would have allowed an early detection of T315I mutation (still at low levels at the beginning), and would have shown how the resistant clone became selected and progressively raised during the TKIs treatment.

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THE TREATMENT OF CHRONIC MYELOID LEUKEMIA BY IMATINIB MESYLATE GENERIC: ABOUT 26 CASES
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Background. Chronic myeloid leukemia (CML) is a chronic myeloproliferative disorder. It is characterized by the presence of chromosomal translocation (9:22), which is transcribed at chimeric protein tyrosine kinase. Its prognosis has been transformed since the advent of imatinib mesylate (GÉVEC®) a specific tyrosine kinase inhibitor (TKI). Some generics (copies) of this molecule are used in many countries, including Morocco, but no study of their effectiveness has been conducted. Aims. The study of the efficacy of treatment with imatinib mesylate. Methods. A retrospective study conducted between April 2005 and March 2010. Within the Department of clinical haematology of Military Hospital in Rabat (Morocco), this study included all the cases of CML treated by imatinib mesylate generic. Results. Twenty six patients were examined. Their median age was 45.5years (16 to 75 years). The sex ratio H/F was the 0.85. The average time of diagnosis was 3 months (0 to 12 years). The clinical presentation was characterized by splenomegaly in 22 patients (85%). The hepatomegaly was found in 2 patients (8%). In 3 cases (12%), the discovery was coincidental. The leukocytosis was over than 105/mm3 in 16 patients (70%), the hemoglobin was lower than 10 g/dl in 9 patients (35%) and thrombocytosis was detected in 8 patients (31%). The diagnosis was confirmed by conventional cytogenetics in 24 cases (92%) and the molecular biology is performed in 2 cases. Additional abnormalities were found in 2 patients. 21 patients were in chronic phase, 4 in accelerated phase and only 1 in blastic phase. The Sokal score was low in 6 cases and intermediate in 16 patients. All patients received treatment with a generic (copy) of imatinib mesylate, produced in India (400mg per day for those in chronic phase and 600 mg per day for those in accelerated phase or transformation). The treatment was stopped in two patients because of intolerance in one and pregnancy in the other. The complete hematologic response at 3 months was 96% (23/24). The major cytogenetic response at 18 months was 77% (17/22) and the major molecular response was achieved in 2 cases. Two patients progressed to blastic phase and 2 others required the use of treatment with TKI of the second generation after the detection of mutations in the tyrosine kinase domain. Conclusions. Despite the small number of patients and limited resources in our series, our results in terms of hematologic and cytogenetic responses were close to the international series.

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CD 68 EXPRESSION IN PATIENTS WITH HODGKIN LYMPHOMA: DOES IT REALLY HAVE A PROGNOSTIC SIGNIFICANCE?
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Background. Even if classical Hodgkin Lymphoma (cHL) is a curable neoplasia, 20 % of patients die from progressive disease. The international Prognostic Score (IPS) and most reported biomarkers are not sufficient to predict individual patient’s evolution. Tumor-associated macrophages have been associated with an unfavourable outcome. In this study, we compare the CD68 status of patients with refractory or relapsed cHL and patients who achieved a durable complete remission (CR). Methods. Immunohistochemical analysis of CD68 expression was performed on initial lymph node biopsies from 28 patients with refractory or relapsed HL and 27 in persisting CR1 recruited in a single institution between 1995 and 2009. CD68 positive cells were counted in representative areas containing Reed Steinberg Cells (RSC), without fibrosis and necrosis. Three groups were defined based on published data and correlation with clinical groups were realised. Results. In refractory (18) and relapsed (10) cHL, median age was 42.5 years (range: 15-70), 19 patients had advanced disease (stage III-IV). IPS was 0-2 in 10 patients, 3-4 in 15 patients, >5 in 3 patients and in 2 patients IPS was not available. Patients received conventional chemotherapy (23 ABVD, 1 MOPP, 4 MOPP/ABV). FDG-PET was performed in 8 patients; two were negative after two courses of chemotherapy. Ten patients died, 5 from toxic death, and 5 from progressive disease. Eighteen patients are alive (15 in CR treated with progressive disease). CD68 expression was <5% in 2 patients, 5-25% in 10 patients and >25% in 16 patients. Among the 27 patients who achieved and maintained a first CR, median age was 39.5 years (range: 17-61). Seventeen patients had advance stage (III-IV). Sixteen patients had advance stage (III-IV). Sixteen patients had advance stage (III-IV). The remaining patient was treated wit BEACOPP. FDG-PET was available in 7 patients and 5 were negative after two courses of chemotherapy. All patients are alive and in CR. CD68 expression was <5% in 3 patients, 5-25% in 8 patients and >25% in 16 patients. No significant statistical correlation could be found between CD68 positivity and age, stage, IPS, treatment response or overall survival. Because of the small number of FDG-PET evaluation, correlation was not performed. Discussion. In this small retrospective cohort, we could not demonstrate any prognostic impact of macrophages infiltration. We could not correlate with early FDG-PET because of the small number of patients. Prospective evaluation of the CD68 positivity impact has to be proposed in clinical trials.

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HODGKIN’S LYMPHOMA ASSOCIATED WITH NEPHROTIC SYNDROME
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Background. Minimal change glomerulopathy has commonly been associated with Hodgkin’s disease. Method and results. We report three cases of nephrotic syndrome accompanied by renal insufficiency (RI) and Hodgkin’s disease. The first patient is a 26-year old man, who was diagnosed with moderate RI and nephrotic syndrome. Renal biopsy disclosed minimal change nephropathy. He was treated initially with steroids and a partial remission of the nephrotic syndrome has been achieved after 5 months of treatment. The second patient, a 62-year old man, was diagnosed with nephrotic syndrome. The renal biopsy disclosed glomerulonephritis with minimal mesangial proliferation. He was treated with cyclophosphamide and steroids without complete remission. Nephrotic syndrome was followed by acute renal insufficiency. The third patient, a 47-year old man, was diagnosed with nephrotic syndrome. Renal biopsy disclosed minimal change nephropathy without renal insufficiency. Six, nine and five months respectively after the beginning of renal disease, nodular sclerosis Hodgkin’s lymphomas were diagnosed to all the three patients. After chemotherapy (6 ABVD cycles) at all patients, a complete remission of nephrotic syndrome as well as a normalisation of renal function was achieved. Conclusions. An extensive evaluation for a lymphoproliferative disease could be advisable in adult patients developing minimal change nephropathy with steroid resistant syndrome.

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THE SERUM LEVEL OF TARC AND IL6 IN CLASSIC HODGKIN LYMPHOMA
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Background. The citocines and chemokines have become important in the diagnosis and prognostic evaluation of malignant diseases. “Thymus and activation-regulated chemokine”(TARC) is an 8 kDa volume polypeptid. It is expressed in large quantities by Hodgkin and Sternberg-Reed (HSS) cells and the secretion of Interleukin-6 is also observed in HSS cells. Our aim was to investigate the serum level of TARC and IL-6 in classic Hodgkin lymphoma (cHL). Methods. IL-6 and TARC level was measured by ELISA. The results were evaluated by MedCalc statistical programme. Results. The serum level of TARC was defined in 108 (49 active, 59 remission), the IL-6 concentration in 64 cases (20 active and 44 remission) and the major molecular response was achieved in 2 cases. Two patients progressed to blastic phase and 2 others required the use of treatment with TKI of the second generation after the detection of mutations in the tyrosine kinase domain. Conclusions. Despite the small number of patients and limited resources in our series, our results in terms of hematologic and cytogenetic responses were close to the international series.

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16th Congress of the European Hematology Association
1238
BLEOMYCIN INDUCED LUNG TOXICITY IN PATIENTS WITH HODGKIN’S DISEASE TREATED WITH BLEOMYCIN CONTAINING REGIMENS (QATARI PROSPECTIVE)

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Background. Bleomycin pulmonary toxicity (BFT) has been known since the early clinical trial of bleomycin in the last century. Postulated risk factors include cumulative bleomycin dose, reduced glomerular filtration rate (GFR), and raised creatinine, older age, supplemental oxygen exposure. Cigarettes smoking, preexisting lung disease, radiotherapy the mount of hydration patient received. And among those with toxicity the supplemental corticosteroids use and interventions with bronchoscopy may play a role in outcome. Aims. To evaluate the postulated risk factors for Bleomycin Induced Lung Toxicity in patients with Hodgkin’s disease treated with Bleomycin Containing regimens in Qatar. Patients and Methods. From our retrospectively collected data of 72 patients diagnosed with Hodgkin’s disease treated at Al-Amal Cancer Center (QATARI) with bleomycin-containing regimens (ABVD, BEACOPP, STANFORD V) for Hodgkin’s Lymphoma between January 2002 and December 2008, with median follow up of 30 months to identify those with BFT. Results. Eleven out of seventy two patients with Hodgkin’s disease (15%) had BFT, range from mild dyspnea to respiratory failure and edema and radiologically, X-ray/CT ranges from normal to overt fibrosis. There were two deaths out of the treated patients directly attributed to BFT. The median time from the start of bleomycin administration to documented lung toxicity is 5 months (range 3-8 months). The following risk factors were evaluated among patients with BFT the patients age were (16-61 year) with median age of 43 year, gender (eight males and three females), none of the patients with BFT is a smoker, weight upon starting bleomycin in patients with toxicity (62-107kg) with a median of 73 kg. Subtypes of HD (6 patients with nodular sclerosis and 5 with mixed cellularity), stage of the disease (6 patients with stage II, 3 patients stage III, and two patients with stage IV), chemotherapy protocols (ten out of eleven patients with BFT received ABVD while the eleventh patient received Stanford V), the cumulative dose of bleomycin ranged from (68-262 international units) with median of 136 IU. Calculation of Creatinine Clearance revealed that none of our patients had grade 2 or more chronic kidney disease. Radiological evaluation of the chest before starting bleomycin containing regimens by CT scan revealed that none of them had pre-existing parenchymal lung disease or fibrosis. Conclusions. In our evaluation for BFT we found toxicity occurring more in males, younger age group and those with nodular sclerosis and mixed cellularity subtypes. It seems that direct toxicity could happen regardless of the dose, and certain races maybe more susceptible to Bleomycin Pulmonary Toxicity, keeping in mind the small number of patients, we feel that further studies are needed to confirm the above mentioned findings.

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AUTOLYUS TRANSPLANTATION IN REFRACRORY OR RELAPSED HODGKIN’S DISEASE

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Background. Relapsed or refractory Hodgkin’s disease is still a challenging problem for hematologists. The standard management of these patients should include the use of salvage chemotherapy followed by autologous stem cell transplant in patients who are chemotherapy sensitive. Aims. We present our experience in 14 patients with relapsed or refractory Hodgkin’s disease who received platinum based protocols as salvage chemotherapy followed by an autologous transplantation as a consolidation therapy. Methods. Fourteen patients (5 males, 9 females), mean age 19.3 years (17-48) are presented. Histologic subtypes included 1 case of lymphocyte predominance subtype, 1 nodular sclerosis and 2 mixed cellularity. Five CR cases were relapsed and 9 resistant to previous therapies (1 patient radiotherapy; three patients ABVD+radiotherapy; ten patients ABVD). Results. Mobilization scheme was salvage chemotherapy (platinum based protocols) plus G-CSF in 13 patients while the other received only G-CSF. The mean number of these cycles was 2 (1-4; median 3). Responses to these cycles and situation of the disease previous to transplant was 5 (35.7%) complete responses (CR), 7 (50%) partial responses (PR) and 2 (14.3%) no response (NR). In all cases the source of progenitors was peripheral blood. Mean number of aphereses performed was 1.35 (1-2; median 1) and no significant problems were registered during the procedures. In all patients enough number of progenitors could be collected. No other mobilization agents (plerixafor) should be used. All patients were irrevocably conditioned with BEAM protocol. Mean CD34+ cells infused was 6x106/Kg (3-18; median 6). After transplantation 12 (85,8%) patients achieved CR, 1 (7,1%) PR and 1 (7,1%) NR. Both patients with no response to salvage therapy achieved PR after transplantation but one of them early relapsed (4 months). Only 1 additional patient (8,3%) relapsed at 9 months after transplantation. Mean overall survival was 25.3 months (4-55+; median 19.5). Conclusions. In our experience, autologous transplantation is a useful and safe procedure in patients with relapsed or resistant Hodgkin’s disease. Salvage therapy with platinum-based protocols plus G-CSF allowed the collection of enough progenitors and all patients with a low number of aphereses. Overall complete response rates after transplantation is high (including one case not responding to prior salvage therapy) and the number of relapses low. Mean overall survival in our series is, up to now, slightly higher than two years.

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RECOVERY OF SPERMATOGENESIS (SP) AFTER BEACOPP14 (B-14) PROTOCOL IN HODGKIN’S LYMPHOMA (HL) YOUNG MALES

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Background. The B-14 protocol is one of the latest cure strategies in advanced stages of HL. There is no medical information on B-14 and gonadal toxicity in young males. Aims. To evaluate the frequency of sperm disorders and infertility, to measure sex hormone concentrations (LH, FSH, Ts) as well as the number of patients who succeeded in achieving fatherhood with and without assisted reproductive technologies (ART). Methods: from 2006 to 2011, 25 men of mean age 25yrs (18-29) with IIB-IV st of classical HL were examined after cessation of treatment with 6-8 of B-14 and IFI irradiation in some pts with bulky mediastinum. All patients had the established fact of pregnancy (spouse pregnancy) and/or had cryopreservation of sperm prior to treatment. Time from the end of chemotherapy to the beginning of the survey averaged 16 mos (2-42). Results. Recovery of Sp was established in 60% pts (15 of 25) with mean time of recovery 16 mos (2-42). Azoospermia was observed in 40% pts (10 of 25) with mean 17 mos (4-42) of observation. Levels of sex hormones were evaluated in 24 of 25 pts, changes were noted in 71% pts (17 of 24). In Sp recovery group the following trends were observed: Ts decrease and L/H, FSH increase - 1pt, FSH increase- 1, Ts and L/H decrease - 1, Ts decrease, L/H decrease - 3, LH decrease - 1, no changes - 7 pts. In azoospermic group: Ts decrease - 1pt, FSH increase-6, Ts decrease and FSH increase - 2, LH decrease - 1 pt. All patients with recovered Sp have some deviations of Sp: oligozoospermia (O)/astenozoospermia (A)/nekozooospermia (N) in 15/1/9 pts. The number of patients who achieved fatherhood without assisted reproductive technologies (IELF, ART) was 9/5. Of 14 causes azoospermia in 40% of cured young male pts. This fact makes semen cryopreservation before HL therapy a necessary procedure to all reproductive males. Changes in levels of sex hormones (LH, FSH, Ts) are not always associated with azoospermia. Increased FSH levels are most often associated with azoospermia. Low Ts level is not linked with functional impairments. Successful recovery of Sp is always associated with its deterioration. The probability of spontaneous recovery of Sp, improvement of sperm parameters after recovery, as well as necessity of Ts replacement therapy require further study.

1241
TREATMENT OF COMPLICATED AND COMPOUNDS FORMS OF HODGKIN’S DISEASE

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Background. Treatment of Hodgkin’s disease (HD) is well established in “standard”cases; front-line therapy with ABVD or ABVD-like regimens +/- radiotherapy are the cornerstone treatments of disease. The problem appears when HD is associated with another disease; another tumour, infection, autoimmune disease, or when the illness itself relapse after several lines of therapy including bone marrow transplantation. In this cases and “wide”and “creative”management of the disease is compulso-
HODGKIN LYMPHOMA - PROGNOSIS AND EVOLUTION

Aims: We try to describe and explain the management of special forms of HD in which we have to make a decision of treating 2 serious diseases simultaneously with a curative approach, or the specific treatment of HD in an out of protocol approach. Methods: We describe 5 cases of Hodgkin lymphoma of different subtypes: mixed cellularity (2) and nodular sclerosing (3), stage IA to IVB with complicated onset or evolution, in four of this HD was associated with others diseases, the other was a third relapse, after autologous bone marrow transplantation (BMT), without compatible donor. Results: In all cases the initial treatment (alone or combined) was standard dosage ABVD without reduction or discontinuation, in 4 cases was associated simultaneously or sequentially with another/other's treatment's depending on concomitant disease: Case 1 was associated with VIH infection: treatment with antiretroviral therapy was simultaneous with gemcitabine-vinorelbine (GEM-VIN) without because of a PET-TAC positivity after 3rd cycle. Case 2: advanced stage prostate cancer, diagnosed simultaneously and treated with a regimen active for both of them: ABVD (contains an antracycline)+radiotherapy. Case 3: a combined lymphoma treated sequentially with maintenance therapy. Case 4: haemolytic autoimmune anaemia glucocorticoid-resistant treated with rituximab a dosage of 100 mg/week x 4 doses. Case 5 was a relapse of HD after BMT without compatible donor treated with a sandwich regimen: Gemcitabine-vinorelbine(6x) + local radiotherapy + Gemcitabine-cisplatin(2x). (table 1).

Methods. A retrospective study of 85 patients diagnosed with Hodgkin's lymphoma in our Clinic of Hematology. Evaluation criteria of patients were: age, sex, number of lymph node areas affected, determining mediastinal, extranodal determinations, clinical stage of disease, histological type of disease, this splenomegaly, anemia, lymphopenia, serum iron, LDH, ALKP, response to treatment. RESULTS: The study group was found predominance of females. Mediastinal mass were met in 28 patients. Most cases were in stage at diagnosis II / III. Primary extranodal Hodgkin lymphoma affecting lung in one case, a case with pericardial tumor and spinal disease in one case was found.13 cases had onset splenomegaly. In terms of histological type in our study group was found that patients with high ferritin levels at diagnosis tend to have a wide variation with a median of 142 ng/mL and a range between 0.4 and 65 months.  Informed consensus has been signed between 11.3 and 1530 ng/mL. We found a significant correlation between ferritin levels at diagnosis and interim-PET positivity, having patients with an interim-PET assessment levels of ferritin greater than patients with a negative interim-PET (p=0.05). Additionally, we found that patients with high ferritin levels at diagnosis tend to have a reduced PFS compared to subjects with normal or moderately increased ferritin (p=0.06), independently from IPS score (respectively, 17.48 months vs not achieved median). The median follow-up was 17.1 months with a range between 0.4 and 65 months. Informed consensus has been signed according to Good Clinical Practice and Helsinki declaration. Results: Ferritin levels showed a wide variation with a median of 142 ng/mL and a range between 11.3 and 1530 ng/mL. We found a significant correlation between ferritin levels at diagnosis and interim-PET positivity, having patients with an interim-PET assessment levels of ferritin greater than patients with a negative interim-PET (p=0.05). Additionally, we found that patients with high ferritin levels at diagnosis tend to have a reduced PFS compared to subjects with normal or moderately increased ferritin (p=0.06), independently from IPS score (respectively, 17.48 months vs not achieved median). Summary/Conclusion. Taken together, our observations suggest how the evaluation of ferritin at diagnosis can add prognostic information in early stage HL patients helping in suspecting the interim-PET assessment and the response to treatment.

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FERRITEN EVALUATION AT DIAGNOSIS CAN IMPROVE THE CHEMOSENSITIVITY AND RESPONSE ASSESSMENT OF EARLY STAGE HODGKIN LYMPHOMA PATIENTS

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Background: In the present study, we evaluated the role of Ferritin in predicting the interim-PET results and the outcome. Methods: Forty-four patients with early stage HL with unfavorable prognosis were treated with ABVD as first line and PET assessment was performed at diagnosis, after two cycles (interim-PET), and at the end of treatment. PET images were interpreted visually according to Dann et al., 2010 and 5 of them (11.36%) had a positive interim-PET. Response assessment was valuable for thirty patients; three out of five patients with positive interim-PET (60%) progressed or relapsed although their early shift to BEACOPP regimen. Three more patients relapsed although their interim-ET was negative. The median follow-up was 17.1 months with a range between 0.4 and 65 months. Informed consensus has been signed according to Good Clinical Practice and Helsinki declaration. Results: Ferritin levels showed a wide variation with a median of 142 ng/mL and a range between 0.4 and 65 months.  Informed consensus has been signed according to Good Clinical Practice and Helsinki declaration. Results: Ferritin levels showed a wide variation with a median of 142 ng/mL and a range between 0.4 and 65 months.  Informed consensus has been signed according to Good Clinical Practice and Helsinki declaration. Results: Ferritin levels showed a wide variation with a median of 142 ng/mL and a range between 0.4 and 65 months.
ly. Median time to myeloid and platelet engraftment was 22 (17-32) and

RELAPSED/REFRACTORY HODGKIN’S LYMPHOMA

socioeconomic level of the society.

ber of patients, high rates of patients with B symptoms and the lower

results of the DAL/GPOH-HD 95 These results may be due to low num-

hand. The investigation of this switching between the histologic sub-

ous studies, nodular sclerosis was the predominant histologic subtype,

ly - relapsed patients died. The EFS and OS rates at 5 years were 88.4%

period of 10 years (median follow up 40 months) and the autologous

57%). Eleven patients (39%) were allocated into risk group 1, 7 (25%)

were asymptomatic (A; 43%) and 12 had constitutional complaints (B;

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Tandem auto/reduced-intensity t-repleted HAPLOIDENTICAL BONE MARROW ALLOGRAFT FOR

RELAPESED/REFRACTORY HODGKIN’S LYMPHOMA

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Background. Tandem auto/reduced-intensity allograft (auto-SCT/RIC-

allo) is a feasible approach in poor prognosis Hodgkin lymphoma patients (HL). However, a minority of patients have an available matched related or unrelated donor. For those patients, haploidentical donor could be an viable alternative donor. Recently, post-transplantation cyclophos-

phamide (Cy) is effective to prevent graft versus host disease (GVHD) and graft rejection, using T-replete bone marrow. Aims. To investigate feasibility of tandem auto-SCT followed by RIC-haplo with high-dose post-transplantation Cy in poor prognosis HL patients. Patients and Meth-

ods. From January 2009, 6 HL patients were treated with auto-SCT fol-

lowed by RIC-haplo, with high-dose post-transplantation Cy. Patients not in FDC-PET complete remission (CR) after 2 chemotherapy lines were included. No patients were relapsed after previous HDC. High dose chemotherapy (HDC) consisted of Melphalan 200 mg/m2 in 5 patients, and BEAM in 1 patient. RIC-haplo conditioning consisted of fludarabine (50 mg/m2 x 5 days), Cy (14.5 mg/kg/day x 2 days, and TBI (2 Gy). Unmanipulated bone marrow cells were infused (target MNC dose 4x 108/kg of recipient). GVHD prophylaxis consisted of Cy 50 mg/m/kg/day for 2 days (d +3 and 4), and tacrolimus and MMF (starting dosage of 1.5 mg/kg/day). GVHD prophylaxis was successful in all patients. The analysis of cost to detect a single event is high and radiation expo-

sure to detect a single event should be considered. Conclusions. For patients with HL in first disease remission, surveillance PET/CT appears to be expensive, with limited clinical impact.

1247

THE SIGNIFICANCE OF EBV VIREMIA IN KOREAN PATIENTS WITH HODGKIN LYMPHOMA

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Background. Epstein-Barr virus (EBV) is known to be frequently asso-

ciated with Hodgkin’s lymphoma (HL). However the significance of EBV infection regarding the clinical outcomes of HL patients has not been explored yet. Several recent studies have reported the presence of EBV-DNA in blood of patients with EBV-positive HL.

Aims: The aim of this study is to investigate the frequency of detection of EBV-DNA in blood of Korean HL patients and the clinical characteristics of patients with EBV viremia. Methods. Between October 2007 and
May 2010, a total of 34 patients with newly diagnosed HL were tested for EBV-DNA titer in blood before treatment, using real-time quantitative PCR. Results. Among them, 6 (17.6%) patients had a detectable EBV-DNA. In situ hybridization (ISH) for EBV-encoded RNA (EBER) in tumor tissues was performed in 5 of the 6 patients and all of them were positive. EBV-ISH could not be performed in one patient as tissue was not available. Patients with EBV viremia seemed to be older (median 62 years, range 43-76 years), compared to patients without EBV viremia (median 31 years, range 14-77 years). In patients with EBV viremia, the proportions of advanced stage (Ann Arbo stage III-IV, 83.3% vs. 46.4%), extra-nodal involvement (66.7% vs. 39.3%), and international prognostic score (IPS) more than 3 (83.3%, vs. 18.5%) seem to be higher than in patient without EBV viremia. Monitoring of viral load was performed in 43 days. After treatment, EBV-DNA was undetectable in two patients with initially low titers (6250 and 1075 copies/mL), and other 2 patients with relatively higher titers at baseline showed decrease in titers (12250 to 3500, and 14500 to 8000 copies/mL). These 4 patients achieved complete response while the one lost to follow-up and the other 2 patients with relatively higher titers at baseline did not show a response. Furthermore, serial monitoring of EBV-DNA titer may help to predict response to treatment in Korean HL patients.

Aims.

To evaluate the state of disease and 5 years survivals by Kaplan-Meier methods. In all patients was administered chemotherapy (3ABVD) and radiotherapy. No relapse was found to explain ITP (18FDG-PET/CT scan, bone marrow biopsy). ITP was treated with corticosteroid and intravenous immunoglobulin (IVIG) but sustained response was not observed. Romiplostim was efficient at maximal dose. Case 2: 77 years old woman was treated in 2009 for stage IVb HD’s with COOP/ABV. In January 2010 she developed auto-immune haemolytic anaemia and severe bleeding due to ITP (platelets 2.10^9/L, haemoglobin 88g/L). No relapse was found to explain ITP (18FDG-PET/CT scan, bone marrow biopsy). ITP was successful but relapse was observed. Treatment with Rituximab was efficient in 8 weeks. Case 3: A 10 years old man with history of HD staging IV Ab by lung and liver diagnosed 2 years ago (December, 2004), and treated with chemotherapy type OPPA/COAP, associated with radiotherapy with complete response, was affected by ITP. Clinical and biological findings were cutaneous purpura, thrombocytopenia at 50.10^9/L, and a peripheral origin on bone marrow aspiration. secondarly appeared auto immune haemolytic anaemia with warm antibodies with a minimum value of haemoglobin level at 58 g/L. Treatment by corticosteroids was unsuccessful and RITUXIMAB was introduced with an increased of the 2 lineages obtained in few days. Conclusions. auto-immune cytopenias could affect patients with a history of Hodgkin’s disease in the absence of relapse. In all cases cytopenias were resistant to the first level of treatment. Severe symptoms allow us to use major therapy of ITP or AIC as Rituximab or Nplate to avoid life-threatening complications.

References

famideteposide/vincristine (IEV) combination chemotherapy. She proceeded to stem cell mobilisation and collection. Several attempts at that time to biopsy the areas of persistent PET positivity failed because of morbid obesity. She remained otherwise very well and a watch and wait approach was adopted. However, progressive widespread PET positive lymphadenopathy developed and biopsies were again attempted 2 years later. Pathology confirmed extensive non-caseating granulomatous inflammation with lymph nodes containing post-treatment residual high-grade lymphoma. Serum angiogenisin converting enzyme level was normal at 21 IU/L (normal range 8-52 IU/L). Patient 2 is a 72 year old female diagnosed with extensive diffuse large B-cell lymphoma (DLBCL), with bulky para-nasal sinus involvement. She received 8 cycles of rituximab/cyclophosphamide/doxorubinc/vincristine/pradniolone (R-CHOVP) combination chemotherapy with CNS prophylaxis with intrathecal methotrexate. An FDG-PET scan at the end of treatment showed persistent FDG avid mediastinal lymphadenopathy. Endobronchial ultrasound guided biopsy of these lymph nodes was performed and samples from the two nodes biopsied showed non-caseating granulomata. There was no evidence of residual high-grade lymphoma. Serum angiogenisin converting enzyme level was just above the normal limit at 55 IU/L (normal range 8-52 IU/L). Conclusions. FDG-PET scanning is fast becoming a cornerstone in the management of certain lymphomas. However, use of this imaging modality for detecting residual disease during and after treatment may be confounded by other reasons for positivity including sarcoidosis. Biopsy confirmation of suspected residual disease may be important before proceeding to high-dose salvage chemotherapy.

1251 IS COMBINATION OF FOUR CYCLES OF EBEACOPP AND FOUR CYCLES OF BEACOPP APPROPRIATE TREATMENT OPTION FOR PATIENTS WITH ADVANCED STAGE HODGKIN’S LYMPHOMA?
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Introduction. HD-12 trial of the GHSG de-escalated therapy of advanced stage of Hodgkin lymphoma (HL) by comparing 8 cycles of escalated BEACOPP with 4 cycles of escalated BEACOPP and 4 cycles of baseline BEACOPP. We evaluate efficacy and toxicity of this scheme used in our institution since 2001. Patients and methods. A total 78 patients with newly diagnosed HL - advanced stage were treated with this approach between December 2001 and December 2010. 62 of them (median age 31 years, range 18-61 years) with minimal follow-up of 12 months from the end of therapy were finally evaluated. Initial stage II/III/IV disease was found in 12/52/18 patients, respectively. We analyzed this group of patients for early toxicity, outcome in interim restaging and treatment outcome. Results: A total of 59 (95 %) patients achieved complete remission. One patient progressed on treatment, one had stable disease. One patient died during treatment. Radiotherapy was given to 10 patients with residual PET positivity. Three patients experienced disease relapse 13/22/22 months after the beginning of the therapy. Relapse or progression occurred in only 5 patients (8%). With the median of follow-up (FU) 59.5 months the FTF for all patients is 92 %, OS is 93 %. Toxicity: Pre-planned 8 cycles of the therapy completed 54 patients (87%). 5 patients didn’t complete treatment due to adverse events (AE), 2 due to non-compliance. Major toxicities were hematologic. The grade 3-4 anemia has occurred in 26 patients (42 %), grade 3-4 neutropenia in 55 (89 %) and grade 3-4 thrombocytopenia in 23 (37 %) patients. G-CSF support in baseline BEACOPP was needed in 41 (66 %) patients, 9 patients were hospitalized due to febrile neutropenia. Other examined early adverse events were aseptic necrosis of the hip of venous thrombosis, soft tissue absces, chronic osteomyelitis, peripheral neuropathy, pneumoni- tos and osteoporosis. During the FU period 1 patient had secondary malignancy, 1 patient myelodysplasia following salvage high-dose chemotherapy in cell rescue. Conclusions. These data showed that combination of escalated and baseline BEACOPP chemotherapy seems to be effective treatment with acceptable acute toxicity, very promising effectivity. Longer follow-up is needed for evaluating of late toxicity of this regimens.

1252 PATIENTS WITH MYELODYSPLASTIC SYNDROMES SHOW REDUCED FREQUENCIES OF CD4+CD8+ DOUBLE POSITIVE T-CELLS
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Background. Even though the expression of CD4 and CD8 on thymocytes is considered mutually exclusive, CD4+CD8+ double positive T-cells (DP) represent a small subset of T-lymphocytes which have been described in the peripheral blood of normal individuals as well as in some pathological conditions. In particular an age dependent accumulation of monoclonal DP has been shown in elderly healthy subjects. From the functional point of view DP are able to act as differentiated effector cells with specific functional activities. However, considering the concomitant expression of granzyme B, Foxp3, interleukin 17 as well as of both Th1-type and Th2-type cytokines, it is very likely they are able to mediate different functions, not only in the peripheral blood but also in other sites. Aim and methods. Although a DP cell population has been found in the lymph nodes of patients with non-dominant Hodgkin’s lymphoma, the relative representation of this cell subset has never been analyzed in patients with myeloid malignancies. As myelodysplastic syndromes (MDS) are a group of clonal disorders characterized by a marked immune dysregulation specifically involving the T-cell compartment, we evaluated the frequency of DP in the peripheral blood of patients with MDS and 40 age-matched normal controls using flow cytometry. Results: We showed that MDS patients when compared with normal controls had reduced frequencies of DP (0.99% ± 0.72% vs 1.38% ± 0.80% calculated on total lymphocytes; p<0.05). We then looked at the possible impact on DP frequencies of several patient- and disease-related factors but, after stratifying patients by WHO adapted Prognostic Scoring System (WPSS), cytogenetics, age, sex, time since diagnosis, neutrophil and platelet counts, transfusion dependence and coexistence of autoimmune phenomena, we could not detect any statistically significant difference. However by comparing DP frequencies in patients belonging to different WHO subcategories, we demonstrated a further reduced frequency of DP in patients with refractory cytopenia and multilineage dysplasia (CRDM) than in patients belonging to the other WHO subcategories (0.98% ± 0.43% vs 1.00% ± 0.68%; p<0.005). Conclusions. Our data further suggest that an abnormal activation of the T-cell compartment may be deeply involved in the pathophysiology of MDS, especially in subtypes such as RCMD which are more likely characterized by the functional inhibition of hematopoietic precursors mediated by auto-aggressive T-lymphocytes described in these disorders. The very recent evidence that Myb is a fundamental promoter of DP survival, along with the demonstration that this gene is typically down-regulated in MDS, could well explain the reduced frequency of DP we observed in our patients.
MoDC had a reduced ability to stimulate allogeneic donor T-cells but the results were compared to 9 normal controls which were cultured in the autologous (from the same patient) and allogeneic (donor) T-cells. After expression of co-stimulatory and activation antigens (CD80, CD86, CD11c (p=0.03), CD80 (p=0.05) and CD86 (p=0.03) was reduced in 4 out of 120 healthy volunteers donors by flow cytometry. Flow cytometry-based analysis of peripheral blood T cell subsets was performed using a whole blood lysis technique in order to avoid the potential loss of certain T cell subsets. Lymphocyte counts were calculated from the white blood cell count and the percentage of lymphocytes determined by flow cytometry. The monoclonal antibodies (mAbs) used in this study were as follows: anti-CD3 (SK7); anti-TCR-α-β-1 (WT31); anti-TCR-γ-δ-1 (11F2); anti-V6-1 (TS8.3); anti-V6-2 (Immu389); anti-NKp46 (9E2/NKp46); anti-CD86 (MEM185); anti-CD95 (FAS1, CD95L, DX2); control mouse IgG1-PE (679.1M7); mouse IgG1-FITC (DAK-G01) and mouse IgG1-PerCy5.5 (X40). Activation-induced apoptosis and expansion of the γδ T cells in vitro were also analyzed. Expression of Intracellular Bcl-2 family proteins was examined by staining the permeabilized cells with anti-Bcl-2 and anti-Bim mAbs. This study was approved by the institutional review board at Akita University. Results: The average numbers of T lymphocytes in blood from all donors examined (n=120) were as follows: 1,084 ± 369 (SD) αβ T cells, 68 ± 44 γδ T cells, 16 ± 12 Vβ1 T cells and 43 ± 36 Vβ2 T cells (μL). The absolute numbers of γδ T cells were reduced in association with aging (R=0.378, p<0.001). The decrease of γδ T cells was a result of age-related decrease of Vβ2 T cells (R=-0.419, p<0.001) but not of Vβ1 T cells (R=-0.098, p=0.299). As a result, the Vβ2/Vδ1 ratio showed an age-dependent decrease. Gender also affects the γδ T cell repertoire, and the numbers of Vβ2 T cells were significantly higher in male donors than female donors (p=0.007). The numbers of αβ T cells, Vβ1 T cells and NK cells were significantly higher in CMV-seropositive donors than CMV-seronegative donors. The Vβ2 T cells but not Vβ1 T cells showed a rapid reduction in cell numbers against mitogen stimulation, and exogenous addition of IL-2 did not rescue the Vβ2 T cells to die. Annexin-V binding was increased at 6 hours of PHA stimulation in all T cell subsets, and the Vβ2 T cells showed the strongest Annexin-V binding. Bcl-2 protein expression was down-regulated in mitogen-stimulated Vβ2 T cells but not in Vβ1 T cells. Summary/conclusions: These results indicate that age and gender have great impact on the γδ T cell repertoire in Japanese donors as well as European and American donors. Age-related decrease of Vβ2 T cells might be explained by their susceptibility to activation-induced cell death.

**1254**

**MATURE MONOCYTE DERIVED DENDRITIC CELLS HAVE ABNORMAL ANTIGEN EXPRESSION BUT APPEAR TO STIMULATE AUTOLOGOUS CD8+ T-CELLS IN MDS PATIENTS**

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**Background:** An activated immune environment has been described in MDS and it is hypothesized that T-cells are reacting to antigens present on the surface of the malignant cells leading to inhibition of colony growth and apoptosis. However it has been demonstrated that selected CD84+ cells in the presence of cytokines and the absence of accessory cells in the bone marrow have colony growth similar to normal. It has therefore also been suggested that T-cells are not solely responsible for the characteristic cell death observed in the bone marrow and that other cells such antigen presenting cells could also play a role. Dendritic cells have been shown to be clonal and abnormal in MDS and therefore their defective interaction with T-cells could be important and requires investigation. **Aims:** The aim of this study was to examine the ability of antigen presenting cells to mature, express co-stimulatory molecules and activate both allogeneic and autologous CD4+ and CD8+ T-cells. Methodology: 5 patients with MDS were studied. All patients and normal individuals were required to read and sign a consent form according to University of Cape Town ethical guidelines. Monocyte derived dendritic cells (MoDC) were isolated from peripheral blood monocytes with GM-CSF and IL-4 for 5 days. The immature MoDC were then activated using LPS and TNFα and thereafter analysed for the expression of co-stimulatory and activation antigens (CD80, CD86, CD11c, CD1a) using standard multiparameter flow cytometry. The activated antigen presenting cells were then cultured with both autologous (from the same patient) and allogeneic (donor) T-cells. After 72 hours of culture, CD4+ and CD8+ T-cells were examined for expression of the activation antigen CD69 using standard flow cytometry. The results were compared to 9 normal controls which were cultured in the same manner. Results: After activation with LPS and TNFα the percent-age and mean fluorescent intensity of expression of HLA-DR (p=0.04), CD11c (p=0.03), CD80 (p=0.05) and CD86 (p=0.05) was reduced in 4 out of the 5 patients with MDS when compared to the normal MoDC. CD1a and CD36 expression was however similar to normal. The mature MoDC had a reduced ability to stimulate allogeneic donor T-cells but the activation and CD69 expression of autologous CD8+ and CD4+ T-cells was increased when compared to normal controls. **Conclusion:** This study confirms that antigen presenting cells in myelodysplasia do not mature normally, have reduced expression of co-stimulatory molecules and are unable to effectively stimulate allogeneic T-cells. However, the results also imply that their capacity to activate autologous T-cells is enhanced indicating that that they are able to present antigen. These findings suggest that both T-cells and dendritic cells could be utilised to develop anti-tumour immune based therapies in myelodysplastic patients.

**1255**

**AGE-ASSOCIATED ALTERATION OF GAMMA/DELTA T CELL REPERTOIRE AND DIFFERENT PROFILES OF ACTIVATION-INDUCED DEATH OF V-Delta1 AND V-Delta2 T CELLS**

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**Background:** Although γδ T cells have been suggested to be involved in T-cell autoimmunity disorders, the role of γδ T cells in bone marrow failure syndrome remains largely unknown. Since age and race have been reported to influence the γδ T cell repertoire, appropriate reference data matched for age and race are absolutely required for clinical studies. **Aims:** The principal aim of this study was to establish reference data on γδ T cell repertoires in a healthy Japanese population and to identify the mechanisms shaping the γδ T cell repertoire. **Methods:** We examined the γδ T cell repertoire in 120 healthy volunteer donors by flow cytometry. Flow cytometry-based analysis of peripheral blood T cell subsets was performed using a whole blood lysis technique in order to avoid the potential loss of certain T cell subsets. Lymphocyte counts were calculated from the white blood cell count and the percentage of lymphocytes determined by flow cytometry. The monoclonal antibodies (mAbs) used in this study were as follows: anti-CD3 (SK7); anti-TCR-α-β-1 (WT31); anti-TCR-γ-δ-1 (11F2); anti-V6-1 (TS8.3); anti-V6-2 (Immu389); anti-NKp46 (9E2/NKp46); anti-CD86 (MEM185); anti-CD95 (FAS1, CD95L, DX2); control mouse IgG1-PE (679.1M7); mouse IgG1-FITC (DAK-G01) and mouse IgG1-PerCy5.5 (X40). Activation-induced apoptosis and expansion of the γδ T cells in vitro were also analyzed. Expression of Intracellular Bcl-2 family proteins was examined by staining the permeabilized cells with anti-Bcl-2 and anti-Bim mAbs. This study was approved by the institutional review board at Akita University. Results: The average numbers of T lymphocytes in blood from all donors examined (n=120) were as follows: 1,084 ± 369 (SD) αβ T cells, 68 ± 44 γδ T cells, 16 ± 12 Vβ1 T cells and 43 ± 36 Vβ2 T cells (μL). The absolute numbers of γδ T cells were reduced in association with aging (R=0.378, p<0.001). The decrease of γδ T cells was a result of age-related decrease of Vβ2 T cells (R=-0.419, p<0.001) but not of Vβ1 T cells (R=-0.098, p=0.299). As a result, the Vβ2/Vδ1 ratio showed an age-dependent decrease. Gender also affects the γδ T cell repertoire, and the numbers of Vβ2 T cells were significantly higher in male donors than female donors (p=0.007). The numbers of αβ T cells, Vβ1 T cells and NK cells were significantly higher in CMV-seropositive donors than CMV-seronegative donors. The Vβ2 T cells but not Vβ1 T cells showed a rapid reduction in cell numbers against mitogen stimulation, and exogenous addition of IL-2 did not rescue the Vβ2 T cells to die. Annexin-V binding was increased at 6 hours of PHA stimulation in all T cell subsets, and the Vβ2 T cells showed the strongest Annexin-V binding. Bcl-2 protein expression was down-regulated in mitogen-stimulated Vβ2 T cells but not in Vβ1 T cells. Summary/conclusions: These results indicate that age and gender have great impact on the γδ T cell repertoire in Japanese donors as well as European and American donors. Age-related decrease of Vβ2 T cells might be explained by their susceptibility to activation-induced cell death.

**1256**

**SIMULTANEOUS DETECTION OF GENOMIC REARRANGEMENTS IN MYELODYSPLASTIC SYNDROMES (MDS) USING THE MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) ASSAY**

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Cytogenetic analysis of the bone marrow is indicated in MDS not only for diagnostic purposes, but also to assess individual prognosis and to tailor the IPS5 scoring system to individual therapy. Conventional cytogenetic (Ck) analysis is performed in clinical practice to detect chromosomal abnormalities. It has been reported that fluorescence in situ hybridization (FISH) is a more sensitive approach, however this analysis is limited to detection of the more frequent abnormalities on chromosomes 5, 7, 8, 11, and 20, and conflicting data are reported in literature. A new method has recently been described for the meas-
The CC study was performed following standard protocols and at least 20 metaphases were analyzed. Our study showed a good correlation between the MLPA and CC results (Table 1), since most of the alterations being detected by both techniques. Discrepancies were found in 7 samples (18.5%). MLPA analysis did not detect the presence of a chromosomal rearrangement (sample n°4); a chr. deletion and a chr. translocation (sample n°11); a chr. deletion (sample n°15); several chr. translocations and deletions (sample n°20). In fact, the MLPA assay is not able to detect chr. translocations but only chr. loss or gain; it can only analyse the chr. regions commonly involved in MDS: 5q (9 probes), 5p (1 probe), 7q (8 probes), 7p (2 probes), 8q (8 probes), 8p (2 probes), 11q (8 probes), 12p (6 probes), 17q (2 probes), 17p (4 probes), 20q (5 probes), 20p (1 probe) and 21q (5 probes). The MLPA was performed on all samples according to the manufacturer’s recommendations (MRC-Holland).

The CC analysis aimed to be performed following standard protocols and at least 20 metaphases were analyzed. Our study showed a good correlation between the MLPA and CC results (Table 1), since most of the alterations being detected by both techniques. Discrepancies were found in 7 samples (18.5%). MLPA analysis did not detect the presence of a chromosomal rearrangement (sample n°4); a chr. deletion and a chr. translocation (sample n°11); a chr. deletion (sample n°15); several chr. translocations and deletions (sample n°20); a chr. gain (sample n°27). In fact, the MLPA assay is not able to detect chr. translocations but only chr. loss or gain; it can only analyse the chr. regions commonly involved in MDS: 5q (9 probes), 5p (1 probe), 7q (8 probes), 7p (2 probes), 8q (8 probes), 8p (2 probes), 11q (8 probes), 12p (6 probes), 17q (2 probes), 17p (4 probes), 20q (5 probes), 20p (1 probe) and 21q (5 probes). The MLPA was performed on all samples according to the manufacturer’s recommendations (MRC-Holland).
was proportional to deviation degree. Considering deviations from normal profile, defined by mean value ± two standard deviations. The phenotypic data were also displayed through a color code (Figure 1): black corresponded to normal expression; red and yellow corresponded to mild and severe aberrancies, respectively. Phenotypic aberrancies were quantified by the percentage of positive cells within a compartment and/or mean fluorescence intensity (MFI; arbitrary relative linear units, scaled from 0 to 104). Phenotypic aberrancies were quantified by the percentage of positive cells within a compartment and/or mean fluorescence intensity (MFI; arbitrary relative linear units, scaled from 0 to 104). Infinicyt (Cytognos) software was used. Our approach was adapted from what described by Matarraz et al (Leukemia 2008). Some major BM compartments were identified as: RA, 7 patients; RARS, 1; RCMD, 5; RAEB-1, 8; RAEB-2, 4. CD34+ cells of B12 def showed no significant phenotypic difference from controls. Conversely, neutrophil compartment had several deviations from normal pattern. As highlighted in Figure 1, SSC resulted significantly higher for B12 def (median value 291 vs 226, p=0.011); this finding appeared more evident restricting the analysis on stage-IV mature granulocytes (295 vs 250, p=0.0002), likely expressing the phenotypic counterpart of hypersegmented neutrophils. Moreover, in MDS subset, stage-IV granulocytes showed a significant reduction of SSC in a substantial fraction (24%) of patients, as expression of hypogranularity and pseudo-Pelger abnormality. Monocytic lineage showed an increased FSC for B12 def (560) compared to controls (462, p=0.0006) without any other relevant difference. Erythroid subset was featured by several alterations, primarily regarding an increase on global cellularity (50.5% vs 9.9%, p=0.0002). The cells revealed higher FSC (253 vs 226, p=0.0002) and SSC (30 vs 20, p=0.0064), while the antigenic profile showed a weaker CD236 (125 vs 174, p=0.04) and CD71 (1077 vs 3207, p=0.0245). As depicted in Figure 1, the phenotypic aberrancies of B12 def within the erythroid compartment resembled what found in MDS. Summary/conclusions. Through a multiparameter and systematical approach, we highlighted some aberranices shared by patients with B12 def and defined a useful profile to distinguish it from clonal disorders.

1259 MARROW CELLS OF VITAMIN B12 DEFICIENCY CASES SHOW A PHENOTYPIC PROFILE SIGNIFICANTLY DIFFERENT FROM MYELODYSPLASTIC SYNDROME

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Background. The diagnosis of myelodysplastic syndromes (MDS) relies on documentation of morphologic dysplasia in bone marrow (BM) cells. Many reports have proposed flow cytometry (FC) as an useful integrating technique in this subset. Nonetheless some non-malignant disorders, such as vitamin-B12 deficiency (B12 def), mimic MDS causing macrocytic anemia and megaloblastic-like abnormalities. Aims. We compared the immunophenotypic characteristics of B12 def patients’ BM cells to normal BM and MDS samples. Our aim was to verify if FC was able to differentiate between the diseases and to identify any phenotypic feature useful to suspect a deficiency-related pathogenesis. Methods. 2x10⁶ BM cells were stained with quadruple combinations of a wide panel of monoclonal antibodies; 50,000 events of global BM cellularity were acquired; if required, a second step was performed to collect 1x10⁶ CD34+ cells at least. Data acquisition was performed using FACSCalibur flow cytometer and CellQuestPro software (Becton Dickinson). For data analysis, Infinicyt (Cytognos) software was used. Our approach was adapted from what described by Matarraz et al (Leukemia 2008). Some major BM compartments were identified on the basis of forward (FSC) and sideward (SSC) light-scatter and reactivity for CD45/CD34. Sixty-nine phenotypic parameters were expressed as percentage of positive cells within a compartment and/or mean fluorescence intensity (MFI) of arbitrary relative linear units, scaled from 0 to 104. Phenotypic aberrancies were quantified considering deviations from normal profile, defined by mean value ± two standard deviations. The phenotypic data were also displayed through a color code (Figure 1): black corresponded to normal expression; red and green to higher and lower than the mean, respectively; color intensity was proportional to deviation degree.

Results. BM of 5 patients with proven B12 def were analyzed, and 25 newly-diagnosed MDS patients; according to WHO, they were classified as: RA, 7 patients; RARS, 1; RCMD, 5; RAEB-1, 8; RAEB-2, 4. CD34+ cells of B12 def showed no significant phenotypic difference from controls. Conversely, neutrophil compartment had several deviations from normal pattern. As highlighted in Figure 1, SSC resulted significantly higher for B12 def (median value 291 vs 226, p=0.011); this finding appeared more evident restricting the analysis on stage-IV mature granulocytes (295 vs 250, p=0.0002), likely expressing the phenotypic counterpart of hypersegmented neutrophils. Moreover, in MDS subset, stage-IV granulocytes showed a significant reduction of SSC in a substantial fraction (24%) of patients, as expression of hypogranularity and pseudo-Pelger abnormality. Monocytic lineage showed an increased FSC for B12 def (560) compared to controls (462, p=0.0006) without any other relevant difference. Erythroid subset was featured by several alterations, primarily regarding an increase on global cellularity (50.5% vs 9.9%, p=0.0002). The cells revealed higher FSC (253 vs 226, p=0.0002) and SSC (30 vs 20, p=0.0064), while the antigenic profile showed a weaker CD236 (125 vs 174, p=0.04) and CD71 (1077 vs 3207, p=0.0245). As depicted in Figure 1, the phenotypic aberrancies of B12 def within the erythroid compartment resembled what found in MDS. Summary/conclusions. Through a multiparameter and systematical approach, we highlighted some aberranices shared by patients with B12 def and defined a useful profile to distinguish it from clonal disorders.

1260 THE SIGNIFICANT ASSOCIATION BETWEEN PRIMARY MYELODYSPLASTIC SYNDROME AND SINGLE NUCLEOTIDE POLYMORPHISMS

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Background. Myelodysplastic syndrome (MDS) represents a heterogeneous group of clonal disorders with ineffective hematopoiesis and its etiology has not been fully explained. Additionally, MDS may be induced by some chemotherapeutic toxins or mutagenic environment factors but the association with certain genes has yet not been detected. Aims. To expand the understanding of MDS etiology we sought to identify genes and polymorphisms associated with MDS using multiplex genotyping. Methods. We used the Illumina Cancer SNP Panel containing 1421 single nucleotide polymorphisms (SNPs) derived from 408 genes thought to be involved in cancer. We conducted a case-control study of 189 patients with primary MDS and 262 controls in Czech population. Firstly, the quality control for all SNPs and samples was implemented and then, Chi² p-value, odds ratio (OR) and upper and lower limits of 95% confidence interval of OR were calculated. When applying Bonferroni correction for multiple testing, the p-value is supposed to show significant association of SNP with the phenotype. Results. Ten SNPs showed significant case-control differences at the level of p<0.001. Findings included an increased risk associated with variants in the anion exchange gene SLC4A2 (p-value=0.0000000001; OR=2.64, 95% CI=1.97-3.55), two ATP-binding cassette transporters genes ABCB1 and ABCCB2 (p-value=0.0000000000; OR=3.85, 95%; CI=2.08-5.48) and p-value=0.0000000000; OR=2.00, 95%; CI=1.20-3.34). The DNA ligase I LIG1 (p-value=0.0000000000; OR=2.10, 95%; CI=1.57-2.82) the aurora kinase AKT6 (p-value=0.0000003; OR=2.51, 95%; CI=1.70-3.71), the progesterone receptor PGR (p-value=0.0000000002; OR=2.14, 95%; CI=1.54-2.92), DNA mismatch repair protein MSH3 (p-value=0.000008; OR=6.71, 95%; CI=2.49-18.08) and the DNA repair gene RAD52 (p-value=0.000006; OR=1.88, 95%; CI=2.58-3.55) and decreased risk associated with the ROS1 gene (p-value=0.000002; OR=0.44, 95%; CI=0.31-0.61). These findings are biologically plausible since association of SNPs in SLC4A2, ABCB1, STK6, LIG1, GPX3 and RAD52 genes with some kind of cancer were described. Conclusions. We observed a significant association between MDS and the common genetic variants described above. This evaluation of genetic polymorphisms identifies SNPs which may be involved in the pathogenesis and biology of this disease. Specific genes associated with risk may have particular relevance for gene function and/or carcinogenesis.

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1261 MYELODYSPLASTIC SYNDROME. EGYPTIAN EXPERIENCE

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Background: Myelodysplastic syndromes (MDS) incidence is unclear because of historical lack of population-based registration and possibly because of under diagnosis. Purpose: To present some retrospective data...
on the epidemiology of myelodysplastic syndrome (MDS) in Egypt, as reflected by a single center based Registry which is the largest tertiary referral center in Egypt. Patients and Methods: Patients diagnosed with MDS and referred to Clinical Haematology unit of Internal Medicine Department Cairo University, Egypt between 2007-2010 were identified. Complete demographic and clinical data, laboratory results, treatment modalities were collected and analyzed. Results: 69 patients with MDS were identified. 39 (57%) females, 30 (43%) male. Mean age was 55 years. 9 (13%) patients were positive for HCV. Mean ferretin level was 544 ng/ml and mean blood transfusion units were 12 units. 26 (38%) patients were RA/CDM, 15 (22%) patients with RA-EB, 10(14%) patients with hypoblastic MDS and 18 (26%) patients with RA and RARS. 12(17%) patients were less than 40 years, 4 (5%) of them had RA-EB. There were a strong correlation between feretin and ALT (r=0.415 P:0.002), ferretin and blood units (r=0.26 P:0.046) and negative correlation between feretin and age (r=0.27 P:0.08). 48 (70%) patients were from rural areas. Summary and Conclusion: We consider this number of diagnosed MDS is a large one and reflects the increased awareness of the disease and improved methods of diagnosis. The young age of diagnose and appearance of RAEB in young patients may reflect the impact of environmental pollution especially water and soil (70% were from rural areas) on the development of genetic mutation. Iron overload is a prominent feature of MDS. The higher prevalence of HCV is part of the problem of chronic hepatitis among Egyptians and could be related to blood transfusion during course of treatment though the strict measures in blood banks. The correlation between ALT with feretin reflected the impact of under treatment of those patients with iron chelation therapy on progression of liver disease especially in presence of HCV.

1262 INTRAVENOUS IRON SUPPORT VS ORAL LIPOSOMAL IRON SUPPORT IN PATIENTS WITH REFRACTORY ANAEMIA TREATED WITH EPOETIN. MONOCENTRIC PROSPECTIVE STUDY

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Background. Intravenous iron support simultaneous to erythropoietin administration improve response to erythropoietin in myelodysplastic patients. In fact intestinal absorption of common commercial oral iron compounds is considerably impaired. Moreover, in MDS patients, absorbed iron is frequently stored in tissues and is not bioavailable. Oral liposomal iron, bypassing normal intestinal mechanism of absorption, shows an increased haematic absorption, better than usual commercial oral iron compounds. Aims. Aim of this study is to verify if in MDS patient support with oral liposomal iron is not inferior to iv iron support. Methods. Between july 2008 and december 2010, 24 patients affected by refractory anemia were studied. Median follow-up was 12 months (R10-24). Patients were randomized 1:1 to receive in group A a sodium ferrugionate 62.5 mg iv in NS 100 ml in 1 h/day in the day when patient received α-erythropoietin 4000 IU sc/week + calcium levonolizate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group B patient received liperox 14 mg 2 tablets orally/day + α erythropoietin 4000 IU sc/week + calcium levonolizate 7.5 mg/day orally + Vitamin B12: 400 mg/day orally. In group A median age was 70 years (R65-75); M/F: 6/8. In group B median age was 66 years (R60-70); M/F: 6/6. Carotype was normal in group A and B patients. Median level of haemoglobin was 9 g/dl in group A (R8.5-11) and 8.8 g/dl (R8.5-11.5) in group B. Results. Group A patients increased Hb level of 1 g/dl after a median time of 4 weeks (R4-7) and after a median time of 5 weeks (R4-8) in group B. Most frequent side effects in group A were erythema in site of injection in 4 patients (35%), hypotension in 1 patient (8%). Most frequent side effects in group B were grade 2-3 diarrhoea in 4 patients (33%). During median follow-up time patients of A and B group gained near 3 g/dl of Hb. Summary/Conclusions. Oral liposomal iron supporting erythropoietic therapy seems to be safe, feasible and substantially not inferior to intravenous iron support in patients affected by refractory anemia.

1263 ASSOCIATION OF MONOClonAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND PAROXYSMAL NOCTURNAL HEMOGLOBINURIA WITH A SMALL CLONE: A CASE REPORT

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Background. Paroxysmal nocturnal haemoglobinuria (PNH) is a haematologic disorder characterized by a clonal expansion of haemato poetic stem cells bearing a deficiency of the glycoprophatidylinositol (GPI-) linked proteins due to an acquired mutation. Usually PNH clones are detected in patients with aplastic anaemia although non systematically they are rarely observed in monoclonal gammopathies of undetermined significance (MGUS). Here is reported the case of a 77-year old woman with both a MGUS and a small PNH clone which remained steady during several years Case report : A monoclonal IgG lambda gammapathy with a PNH clone was diagnosed in 2001. In 2008, plasma cytosis was observed on the bone marrow smear and there were no clinical or radiologic symptoms of myeloma. Methods : Three markers on red blood cells (RBC) were studied by Flow cytometry (CD55, CD59 and CD55) and also two markers on granulocytes (CD55 et CD59) and one on monocytes (CD14). The direct antioglobulin test (DAT) was performed on RBC by gel filtration. Results: In April 2008, CD55-, CD59- and CD59- RBC were at 2.9%, 2.1% and 0.6% respectively. On GN, CD55- and CD59- cells were at 0.9% and 1.8% and on MN, CD14- cells at 5.4%. The haemoglobin (Hb) level was at 94g/L, the haptoglobulin and total bilirubin levels were normal at 0.9 g/L and 9.0 micromole/L respectively. The DAT was negative. In July 2009, the size of the PNH clone was still low (CD55-, CD59- and CD59- RBC were at 1.8%, 0.6% and 0.6%; CD55- and CD59- GN at 2.8% and 1.9%); CD59- MN was at 1.9%. The Hb level was at 107g/L, the lactate dehydrogenase (LDH) level at 380 IU/L, total bilirubin level at 6 micromol/L, the DAT was negative but the haptoglobin level decreased to 0.2 g/L (normal higher than 0.7g/L). In April 2010, the same pattern was observed on PNH cells : CD55-, CD59- and CD59- RBC were at 0.7%, 0.6% and 0.6%; CD55- and CD59- GN at 1.7% and 1.5% respectively; CD14- MN at 2.0%. Conclusion: In April 2010, the same pattern was observed on PNH cells : CD55-, CD59- and CD59- RBC were at 0.7%, 0.6% and 0.6%; CD55- and CD59- GN at 1.7% and 1.5% respectively; CD14- MN at 2.0%. The sign of haemolysis was noted, only a low level of haptoglobin (0.2 g/L). Nevertheless, in June and December 2010, CD14- MN began to increase to 6.4 and 10.1% without change for the other blood cells. Summary/conclusions. This case combines an unusual association of two blood disorders. When first observed the size of the PNH clone was low for several years without sign of haemolysis but with a low level of haptoglobin. No sign of myeloma has been observed during the clinical course.

1264 LOW RISK MDS: 5-AZACITIDINE, WHAT’S ELSE?... LENALIDOMIDE!

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Alternative treatments are poor and limited effect, in low risk MDS patients. However, everybody have some younger patients, that they could receive 5-azacitidine or lenalidomide when it failed. Patients: We reported 4 patients have been treated with 5-azacitidine, they researched a good enithroid response, but they lost it. In this moment, we offer these two chelation therapy with lenalidomide. Case 1: Male 45 years-old was diagnosed of SRA (low IPSS, normal cytogenetic) 5 years ago. He had a transitory respond to EPO. 6 months later, he received 5-aza with a good respond 4 months later. This was mantained to nine cycles. Later, he has initiated Lenalidomide (initial dosage was 10 mg/d, actually he intakes 5mg/48h), he has managed the independent transfusional. Initial Hb was 4.8 g/dl, and now Hb is 10 g/dl, after 6 months of treatment. Case 2: Female 38 years-old was diagnosed of SRA (low IPSS, normal cytogenetic) 2 years ago, she had a transitory respond to EPO. 16 months later, she began 5-aza therapy, with good respond 2 months later. She did keep up to 14th cycle. Later, she has initiated Lenalidomide at dosage 10 mg/d, actually she intakes 5mg/48h), she has managed the independent transfusional. Initial Hb was 6.0 g/dl, and now Hb is 11 g/dl, after 10 months of treatment. At present, she has Hb 9.8 g/dl after 12 months of therapy. Case 3: Male 78 years-old diagnosed of SRA (low IPSS, normal cytogenetic) 1 year ago. He had a transitory respond to EPO. 2 months later, he began 5-aza therapy, with good respond 3 months later. He did keep up to 15th cycle. Later, he has initiated Lenalidomide at dosage 10 mg/d, he has managed the independent transfusional. Initial Hb was 6.0 g/dl, and now Hb is 9.8 g/dl after 12 months of therapy. Case 4: Female 63 years-old was diagnosed of SRA (low IPSS, normal cytogenetic) 20 years ago. She began 5-aza therapy, with good respond 3 months later, and this was maintained 24 months later. Later, she has initiated Lenalidomide at dosage 10 mg/d. At present, she hasn’t respond yet, after 5 months of therapy. Results: The three patients reached the independent transfusional, but we were
obligated to tapering dosage and we must wait 6-10 months to research the response. We reported hematologic toxicity: G3-4 neutropenia and thrombocytopenia. We used G-CSF treatment by 48-72h. Conclusions. 1) In low risk MDS patients, non 5q-, lenalidomide is an alternative therapeutic, when 5-aza has failed; 2) Lenalidomide used dosage, is lower than recommended, by hematological toxicity; 3) The response, if it happens, is later than we hope (between 6-10 months).

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A CASE REPORT OF WHIM SYNDROME (MIELOKATHEXIS) IN BRAZIL - CLINICAL FEATURES AND BONE MARROW MORPHOLOGY

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Background. WHIM syndrome is a very rare congenital disorder with approximately 40 cases described until now. This syndrome is characterized by severe chronic neutropenia, retention and apoptosis of mature neutrophils in the bone marrow (myelokathexis), hypogammaglobulinemia and recurrent bacterial infections. Aim. To report a case of WHIM syndrome in a brazilian patient.

Methods. Clinical and key laboratory data were recorded during the follow up of the patient before and after treatment. Results. A 2-year old girl was evaluated at our hematology clinic with a past history of recurrent infections (5 episodes of pneumonia and 5 episodes of urinary tract infection) and persistent low neutrophil counts, ranging from 64 to 650/μL since she was 10 months old. There were no abnormalities on physical examination. Investigational tests showed hypogammaglobulinemia (gamma globulin=0,29g/dL) on protein electrophoresis and the examination of the bone marrow aspirate revealed a hypercellular bone marrow with granulocytic hyperplasia, characterized by an increased number of mature neutrophils with hypersegmented nuclei and cytoplasmic vacuolization. Neutrophils showed nuclear lobes often separated by long strands of chromatin (Figure 1). These typical bone marrow morphologic findings of myelokathexis in association with the clinical picture were consistent with the diagnosis of WHIM syndrome. Treatment with G-CSF (5mg/Kg per day subcutaneously) was initiated after diagnosis confirmation, leading to an increase in neutrophil count to 550/μL after 12 days of therapy and to 1300/μL after 24 days. No side effects or new episodes of bacterial infection were observed after therapy initiation. Summary/Conclusions. (i). Careful morphological examination of bone marrow aspirate is determinant for diagnosis of myelokathexis, especially in clinical settings where genetic and molecular characterization is unavailable. (ii). Response to G-CSF is in accordance with previous reports.

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CLINICAL AND BIOLOGICAL CHARACTERISTICS, EVOLUTION AND IMPLEMENTATION OF THE NEW PROGNOSTIC SCORE IN A SERIE OF PATIENTS WITH CHRONIC MYELOMONOCYTIC LEUKEMIA (CMML)

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Background. CMML is a heterogeneous clonal hematologic neoplasm with both myeloproliferative and myelodysplastic features. The International Prognostic Scoring System (IPSS) is of limited usefulness. Recently a new prognostic model (Kantarjian et al, Cancer 2008) has been proposed that predicts better survival in these patients. Aims and Methods. Retrospective analysis of clinical and biological characteristics, evolution and application of a new prognostic model for patients with CMML diagnosed in a community hospital between January 2003 to May 2010. Results. Thirty nine patients were identified. They were 28 (72%) males and 11 (28%) women; median age was 75 years (range 55-95). In 18% the performance status measured by ECOG scale was higher than 2. Average and extreme analytical values at diagnosis were as follows: Hb 112 g/L (44-175), WBC 35.1x10^9/L (3.5-82), monocytes 3.6x10^9/L (1-7.9), platelets 151x10^9/L (17-750). At the time of diagnosis, thirteen patients (33%) had leukocytosis >13x10^9/L, 8 (20%) had peripheral blasts (range 2-11), 19 (49%) had melema and 5 (13%) had splenomegaly. Thirty five (90%) of the 39 had CMML type 1 and 4 (10%) had CMML type 2. Eight (20%) patients had cytogenetic abnormalities (+8 (n=2), 11q (n=1), -4 (n=2), -7 (n=2), -5q (n=1)). Seven patients (18%) had previously required blood transfusions. The distribution of patients according to the IPSS was 25 (64%) low risk, 10 (26%) intermediate-1 risk, 4 (10%) intermediate-2 risk and no patient had high-risk IPSS. The distribution according to the score proposed by Kantarjian et al was: 22 patients (56%) low risk, 4 (10%) intermediate-1 risk, 6 (15%) intermediate-2 risk and 8 (18%) high risk. Four patients (10%) of the 39 patients progressed to acute leukemia. Of these, 3 had cytogenetic abnormalities and all showed intermediate-1 IPSS while 1 had a score intermediate-2 or high risk under the new score. With a mean of 23 months (0-84), 19 patients (49%) survived, 17 (43%) have died and over 3 their status is unknown. In 60% the cause of death was secondary to CMML. The medians and ranges of survival (months) of patients stratified by FAB were as follows: MDS-RAEB 0-75 in the AL group; 22 (0-52) in the intermediate-1, 5 (2-6) in the intermediate-2. Applying the new score they were: 22 (0-75) in the low risk, 21 (12-80) in intermediate-1, 16 (0-24) in the intermediate-2 and 5 (2-24) in high risk. Conclusions: The CMML affects elderly patients. Their clinical and biological features are heterogeneous. A minority presents cytogenetic abnormalities. While the small number of patients in this series, the new prognostic score seems to be better than the IPSS stratifying patients into different risk groups.

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TRANSFUSIONS IN HOME CARE PATIENTS WITH MYELODYSPLASTIC SYNDROMES

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Background. The majority of patients diagnosed with myelodysplastic syndromes (MDS) are individuals of older age often afflicted by several comorbid conditions for which they are generally unsuitable for disease-modifying treatments. The treatment of anemia is an essential part of the global management of most MDS patients. In erythropoietin-failed patients or in those unsuitable for this option, red blood cell (RBC) transfusions remain the only available measure. For this category of frail patients and for their families, home care (HC) represent a valuable option allowing to preserve the patient’s quality of life and to avoid useless hospital admissions. Aims. To evaluate the management of RBC transfusions at home during the last two years. Methods. There were 65 MDS (27 male) with a median age of 86 (69 - 98) years. Patients were followed at home for a mean of 8.8 (1 - 24) months. Therapy with erythropoietin stimulating agents was used in 41 pts (60%). Results. Overall, 55 (81%) patients required transfusions, for a total of 927 RBC units; RBC units / transfused pt were a median of 10 (1-68). RBC units monthly requirement in transfused pts was a median of 1.5 (0.05-5.7). A lower baseline Hb concentration and the time for the primary diagnosis of MDS strongly correlated with the number of transfused RBC units. All transfusions were safely administered at home without any untoward effect. Conclusions. Ood. is a particularly important issue for older MDS patients. With this regard, management of chronic patients requiring multiple and repeated admissions to receive RBC transfusions may be a concern for the affected individual and for its family. Our experience demonstrated that the administration of RBC transfusion at home is a feasible, reliable and effective in our older MDS patient, avoiding social
and economic costs due to an inappropriate removal from his domestic environment. In conclusion, in our experience, domiciliary management of RBC transfusions represented an important added value to home care program, allowing the best humanization of this procedure for our patients.

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EVALUATION OF CIRCULATING COLONY-FORMING PROGENITOR CELLS AND TREATMENT RESPONSES IN PATIENTS WITH HLATOMIC ANEMIA: A SINGLE CENTER EXPERIENCE

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Aplastic anemia (AA) is a life-threatening hematopoietic disorder characterized by a bone marrow failure with lesions of granulopoiesis and thrombopoiesis. Standard treatments include hematopoietic stem cell transplantation (SCT) and immunotherapy (IST) with antithymocyte globulin (ATG) and cyclosporin-A (CSA). However, not all patients can be cured with SCT or ATG/CSA. Only a few, more or less robust, prognostic factors predicting long term relapse-free survival in AA are available. We have retrospectively analyzed a cohort of fifty patients with AA (27 males and 23 females) treated in our department between 1987 and 2007. The median age was 37 years (range 14-70 years). Forty two patients received ATG/CSA, and 7 were transplanted upfront using a matched sibling donor. One patient was treated with CSA and growth factors only. In the group of transplanted patients, one patient died from multi-organ failure and 6 are alive and are in continuous complete remission (CR). Of the 43 patients receiving ATG/CSA or CSA, 28 patients (65%) achieved a CR including the one patient who was only treated with CSA, 7 (16%) entered a partial remission (PR), and 8 patients (19%) did not respond to treatment. Eight patients (19%) relapsed after IST. To evaluate possible prognostic factors predicting responses to IST, patient age, karyotype, existence of PNH clones, pre-treatment blood counts, progenitor cell counts, and outcome were evaluated. In this analysis, we found that in IST-treated patients with AA, the numbers of colony-forming progenitor cells increased but remained below the normal range of healthy controls in all patients. We also found that the numbers of granulocytomacrophage colony-forming progenitor cells (CFU-GM) increased during successful therapy and were higher in those who underwent SCT than in patients who received IST (p<0.05). However, we were unable to detect a prognostic risk factor that would predict long term responses or relapse-free survival in these patients, which may be because of the low number of patients examined. In summary our data show that an increase in the numbers of circulating colony-forming progenitor cells is associated with regeneration of bone marrow function in AA patients successfully treated with SCT or IST, although normal CFU levels are not reached. Prospective studies with more patients are required to define prognostic factors predicting relapse-free survival in these patients.

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CYTOLOGICAL FINDINGS OF PAGET’S DISEASE DIAGNOSED BY BONE MARROW ASPIRATION

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Paget’s disease is estimated to occur in 1-3% of individuals older than 45-55 years and in up to 10% in persons older than 80 years. Because early diagnosis and treatment is important, a range of specific imaging and laboratory studies are essential. Clinical diagnosis often is difficult and a bone biopsy may be needed, especially to exclude a metastatic bone disease. Only one case of Paget’s bone disease initially diagnosed by bone marrow aspiration has been reported in English literature. We report the case of a 53-year-old man who presented with vertigo and hypertension of two days duration. On x-ray and CT-scan of the skull osteoblastic bone lesions were noted and a bone scintiscan followed which showed a focal (CPC) play a role on the skull, vertebrae, pelvis and long bones of the lower leg. A bone marrow aspiration was performed and in the aspirate smears were identified multiple osteoblasts mainly in small aggregates and few osteoclasts, findings which were consistent with Paget’s disease. The core biopsy showed an abnormal bony architecture with osteolysis accompanied by osteoblastic bone formation with an increase in the number and activity of the osteoblasts and replacement of the normal marrow with fibrous tissue, findings which confirmed the diagnosis of Paget’s disease. In conclusion, we describe the second case of Paget’s disease diagnosed by bone marrow aspiration which proves to be a relatively simple, cost-effective, and accurate method in the diagnosis of a clinically suspected disease, confirm the radiological impression and may exclude the presence of a malignant process.

1270

5-AZACITIDINE IN PATIENTS WITH MYELODYSPLASIA AND ACUTE MYELOID LEUKEMIA: A SINGLE CENTRE EXPERIENCE

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Background. Hypomethylating agents have recently been shown to prolong overall survival and improve quality of life in patients either with INT-2 and high IPSS risk myelodysplasia (MDS) or with low bone marrow blast count acute myeloid leukemia (AML). Aims. The aim of our retrospective analysis was to evaluate the efficacy and the feasibility of 5-azacitidine therapy in a cohort of patients for whom no alternative therapy is available. Methods. Since September 2008 we have been treating with 5-azacitidine 53 patients affected by acute myeloid leukemia (18 patients), MDS (15 patients) or chronic myelomonocytic leukemia (2 patients). The median age of patients at treatment starting time was 70 years (range: 51-82). Azacitidine was administered subcutaneously (75 mg/m²/d) for 7 days of every 28-day cycle until loss of response or disease progression. Patients received a median number of 6.5 cycles of therapy (range 1-24). According to International Working Group MDS and LMA criteria, in the overall study population we evaluated overall improvement (CR + PR + HI), the best response obtained and adverse events. In the subgroup of patients who received at least 6 cycles of therapy (13 patients) we also evaluated overall survival (OS) and progression free survival (PFS). Results. In the AML cohort, after a median number of 4.6 cycles (range 1-17), we observed a complete response in 8% of patients, a hematological improvement (HI) in 22% of patients, a stable disease (SD) in 39% of patients and a lack of response in 33% of patients. In the MDS cohort, after a median number of 8.5 cycles (range 2-24), we observed a complete response (CR) (including a complete cytogenetic response) in 51% of patients, a partial response (PR) in 7.5% of patients, and a hematological improvement (HI) in 35% of patients and a stable disease in 15% of patients. The overall improvement (CR + PR + HI) was 28% in AML cohort and 84.5% in MDS cohort. In the CMMML cohort, after a median number of 10.5 cycles (range 9-12), we observed a partial response in 50% of patients and a hematological improvement in 50% of patients. In the subgroup of patients who received at least six cycles of therapy, the overall survival was 14 months and the progression free survival was 11.5 months. In the overall study population, only two patients (6%) discontinued treatment as a result of adverse events. Conclusions. The limited number of cases and the short period of follow-up don’t allow us to evaluate overall survival and progression free survival in the whole study population. In the subgroup of patients who received at least six cycles of therapy, we observed that 5-azacitidine plays an important role in treatment of MDS and low bone marrow blast counts AML, particularly with prolonged OS and good safety profile. Further trials should assess the number of cycles required for treatment, the role of hypomethylating agents in low-risk MDS and in patients with AML and a bone marrow blasts counts <30%.

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THE IMPORTANCE OF COMPLEX BLOOD COUNT ASSESSMENT FOR DIAGNOSTICS AND PROGNOSIS IN MDS PATIENTS

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Background. The myelodysplastic syndrome (MDS) manifests as mono-, bi- or pancytopenia in peripheral blood and therefore the full haematology (FBC) plays a key role in its diagnostics and differential diagnostics. The careful evaluation of all parameters of blood count can contribute to fast estimation of MDS prognosis. Aims: The goal of retrospective analysis of 100 MDS patients examined within the period from 2000 - 2008 was evaluation of initial parameters of blood count for the risk of leukemic transformation and overall survival (OS). Methods: Clinical and laboratory data at diagnosis were analysed to assess the prognosis and the risk of leukemia development, were observed the course of disease and overall survival. The cohort consisted of 100 patients (pts), 51 males
5.109/l (Me 1.3), MCV 77 - 125 fl (Me 96), trombocytes 3 - 702.109/l (Me 3,5), neutrophiles 0,1 - 17.10^9/l (Me 1,7), lymfocytes 0 - 5.10^9/l (Me 3,5 - 125 II), platelet mass (MPV) 7 - 125 fl (Me 113), pl mass (MPVx trombocytes) 0 - 6.8 ml/l (Me 1,6), peripheral blasts 0 - 12 % (Me 0). Statistically significant unfavourable prognostic factors for OS showed trombopenia, neutropenia, presence of blast cells in peripheral blood, peripheral blast cells ≥ 5% and low platelet mass. The level of hemoglobin (Hb), MPV and RDW were not shown as statistically significant unfavourable factors. The favourable factors were macrocytosis and lymfopenia. The level of thrombocytes obviously represented only selected cases, the good erythroid and cytogenetic responses suggest that some patients with MDS and del(5q) may benefit from lenalidomide treatment even if this must be discontinued.

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vant chemotherapy treatment were evaluated. A total of 78 patients from Kayser Research and Training Hospital and Mersin State Hospital were enrolled in the study. Their adjuvant treatments had been completed at least 18 months before. No patient had either chronic or infectious diseases. Patients with abnormal ferritin, vitamin B12 or folate levels were excluded from the study. Results. Two patients complained of anemia (2,2%) (Hb<11 mg/dl); leukopenia was observed in seven patients (7,7%) (Leukocyte<4000/mm3); thrombocytopenia was observed in four patients (4,4%) (PLT< 150.000/mm3). It was established that oval macrocytes were 14%, macrocytes 57%, acanthocytes 1%, stomatocytes 12%, teardrops 12%, nucleated erythrocytes 1%, basophilic stippling 14% and Howell-Jolly bodies 1%. There were 36% hypo-granulocytosis, 26% Pelger-Huet abnormality, 20% hypersegmentation, 8% immature granulocytes and 6% blasts. We have established 50% of giant platelets and 19% of platelets hypopigmentation. Discussion. According to the peripheral blood smear assessments of our study, we suggest that breast cancer patients should be evaluated for MDS at the early stage, starting from Month 18, even when the automated blood counts are normal.

1275 PATIENT WITH COPPER DEFICIENCY MIMICKING MYELODYSPLASTIC SYNDROME (MDS)
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Myelodysplastic syndromes (MDS) are a group of heterogenous clonal stem cell disorders characterized by variable degree of cytopenias, cytogenetic abnormalities and evolution to acute myeloid leukemia. In the low risk categories of MDS without cytogenetic abnormalities diagnosis relies on morphology and exclusion of other possible causes of cytopenias such as Vit B12 or folate deficiency. Copper deficiency is usually not taken in account in the study of a possible MDS , several cases of bone marrow failure due to its deficiency have been reported. We present a case of Copper deficiency mimicking MDS. Case report and methods A 65 years old male with anemia (Hb=59gr/L VCM=94) and severe neutropenia (WBC=1.1X10/L, neutrophils=320/mm3) and normal platelet levels was seen at our institution. The patient had progressive gait disturbance. A neurological exam was consistent with subacute combined degeneration as seen in Vit B12 deficiency. Serum levels of B12, folate and ferritin were in the normal range. A bone marrow exam disclosed dysplastic features in granulocyte and erythroid lines with a prominent vacuolization of progenitors of both lines (Picture 1) and 16% ring sideroblasts. A standard cytogenetic analysis did not show any clonal abnormality and FiSH was also negative for del 5q, monosomy 7, trisomy 8 and del 20q. A presumptive diagnosis of MDS refractory cytopenia with multilineage dysplasia of the WHO classification was established. The patient was treated with growth factors with anemia improvement but neutropenia persisted. Due to the vacuolization of progenitors of both lines, all possible mechanism for the anemia associated to copper deficiency was considered. Copper deficiency is usually seen in malnourished patients, parenteral nutrition and after gastrectomy. In some cases, like ours, no cause could be found. Our patient, like others, had hyperzynemia without known exposure, a fact believed by some authors to be an epiphenomenon. Zinc induces metallothionin production in enterocytes for which copper has a great affinity displacing Zinc. Copper is therefore accumulated in gastrointestinal tract and being eliminated with coproporphyrins. All the patients described normal blood counts with copper therapy or Zinc withdrawal but neurological symptoms did not improve. Our patient has just begun treatment to evaluate response but the clinical, morphology and serum levels of Copper and Zinc are concordant with other cases described. Some of the patients described in the literature were detected before a bone marrow transplant. We and others suggest that Copper deficiency should be routinely ruled out before establishing a diagnosis of MDS in the low risk categories especially if they have vacuolated marrow progenitors, ring sideroblasts or neutropathy to avoid potentially harmful therapies.

1276 NEUTROPENIA IN MYELODYSPLASTIC SYNDROMES BASED ON THE DATA OF A SINGLE CENTER ROMANIAN REGISTRY
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Background. The myelodysplastic syndromes (MDS) are a group of clonal disorders with an annual incidence of 4 cases/100000 in the general population. Neutropenia is a common cytopenia in MDS. Aim. The purpose of this study is to evaluate the incidence of neutropenia in MDS and the overall survival of these patients in our department. Methods: this retrospective, epidemiological study incudes 560 patients diagnosed and treated in our department during 1982-2010. Among them 184 cases with neutropenia were found. The registration form was kindly provided by the MDS Foundation (USA) and the diagnosis of MDS was based on well-accepted FAB minimal diagnosis criteria. Neutropenia was defined as a neutrophils count < 1500/dL and was divided into three subgroups of severity (mild 1500-1100/dL, moderate 1000-500/dL, severe <500/dL). The parameters age, sex, rural/urban location, temporal trend and distribution by FAB subtypes were analyzed comparatively in neutropenic and nonneutropenic MDS cases. We also analyzed the complications appeared and the evolution of the disease. Results: the lot includes 184 patients (89 females and 95 males) with an average age of 65 years. The distribution by rural/urban location was grater for urban (77 patients) than for rural location (70 patients). According to FAB criteria, the distribution of neutropenia in MDS patients was: 32.50% RA, 8.69% RARS, 37.50% RAEB, 13.04% RAEB-T, 3.81% CMML and 24.45% U-MDS. Regarding the severity of neutropenia we found 32.06% patients with neutrophils between 1500-1100/dL, 36.41% cases between 1000-500/dL and 31.52% under 500/dL. Severe infectious events were found in 31.60% patients, the site of infection was common-ly pulmonary (42 cases), cutaneous (5 cases) and digestive tract (11 cases). 11 patients required intensive unit care transfers due to acute respira-tory distress syndrome, and 10 died. Overall mortality rate was 15%. Conclusions. Neutropenia in MDS is a challenging finding because, when severe, is an important cause of death by life-threatening infections. The interest in MDS-related neutropenia increases when we are thinking to the economic aspects of its management.

1277 SAFETY AND EFFICACY OF COMBINATION THERAPY WITH DEFERASIROX AND DEFEREXAMINE FOR MANAGEMENT OF IRON OVERLOAD IN MULTITRANSFUSED HEPATOPATIC PATIENT WITH MYELODYSPLASTIC SYNDROME: A CASE REPORT
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Myelodysplastic Syndromes (MDS) is a heterogeneous group of hematopoietic stem cell disorders characterized by cytopenia and hyperplastic bone marrow: in this context red blood cell transfusions represent a life-saving treatment for patients with chronic anaemia. Iron overload is the consequence of a long term transfusion therapy thus it
is necessary to prevent this complication by applying a correct iron chelation therapy. Deferasirox is a well tolerated oral iron chelator drug that produces relevant benefits but, because of its potential hepatotoxicity, it is not recommended for patient with preexisting hepatic diseases. Here we report the case of a 62-year-old man affected by HCV positive cirrhosis and MDS (Refractory Anemia, IPSS 0.5). Recombinant Erythropoietin therapy was ineffective and a RBC transfusion program was started. The patient was on transfusion pro month with a very high ferritin serum concentration near 700 ng/mL. Iron chelation therapy with deferoxamine was proposed in consideration of patient hepatic disease: compliance to subcutaneous injection was very bad, transfusion need increased exponentially until to 2 blood packages per week and serum ferritin concentration reached, in 12 months, the level of 6195 ng/mL. Since high levels of ferritin are associated with a very bad condition for hepatic, and oncologic function was performed. After three months serum ferritin concentration was not modified as well as other biochemical parameters, then deferasirox dosage was gradually increased reaching 20 mg/kg/die after two months and no liver damage was observed. After five months of iron chelation therapy with deferasirox at full dosage serum ferritin concentration remained very high (5098 ng/mL). Then, considering all risks related to transfusion dependent secondary hemochromatosis, with patient informed consent, a combined iron chelation therapy with deferasirox (50 mg/kg/die) and deferoxamine (2 g/day for 5 days/week) was established and after 3 months serum ferritin concentration lowered to 3000 ng/mL. At the present time, the patient receives 2 RBC package pro week and, after two years of combined iron chelation therapy, serum ferritin concentration is at a stable level nearby under 3000 ng/mL. No serious adverse event has been observed. Our suggestion is that combined therapy with deferasirox and deferoxamine could be considered a safe and useful therapeutic choice in the management of critical transfusion dependent iron overload in old MDS patients with hepatic disease.

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MYELODYSPLASTIC SYNDROMES AND CHRONIC MYELOMONOCYTIC LEUKEMIA (ABOUT 23 CASES)

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Background. Myelodysplastic syndromes (MDS) are a set of oligoclonal disorders of hematopoietic stem cells characterized by ineffective hematopoiesis that manifest clinically as anemia, neutropenia, and/or thrombocytopenia of variable severity. Since 2001, the WHO classification considers chronic myelomonocytic leukemia (CMMML) as an intermediate entity between Myelodysplastic and Myeloproliferative syndromes. Aims. The aim of this study is to report the experience of the clinical hematology department, of the Military Hospital Mohammed V, in Maroc, with MDS. This is a retrospective study including all patients who consulted for MDS or CMMML since the creation of clinical hematology department in 2006 until December 2010. The patients with MDS were classified according to 2008 WHO classification. Prognostic scores used are the IWSS and WPSS (CMMML excluded). Response criteria adopted are those of modified IWG Criteria.

Response.

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MYELODYSPLASTIC SYNDROMES WITH DEL(5q) AND JAK2 V617F MUTATION: A CASE OF HEMATOLOGIC RESPONSE TO LENALIDOMIDE THERAPY

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Deletion of long arm of chromosome 5 (del5q) is one of the most common cytogenetic abnormalities in Myelodysplastic Syndromes (MDS). The JAK-2 V617F somatic mutation is the molecular marker most frequently detected in the BCR/ABL negative myeloproliferative neoplasms (MPN). The association between del5q and JAK2 V617F mutation and del5q is an example of MDS successfully treated with Lenalidomide. A 62-year-old woman had been treated with hydroxyurea for 5 years because of a diagnosis of Essential Thrombocytemia (ET) before admission to our hospital for normocytic anemia (Hb 9.5 g/dl) thrombocytosis (PLT 855x10^9/L) and mild leucocytosis (WBC 2.9x10^9/L). Bone marrow aspirate showed increased number of myeloid precursors with dysplastic features, dyserythropoiesis and dysgranulopoiesis, no ringed sideroblast was observed. Cytogenetic analysis performed by Fluorescence in Situ Hybridization (FISH) revealed an isolated interstitial deletion of the long arm of the chromosome 5. Polymerase Chain Reaction (PCR) was positive for JAK-2 V617F mutation. Treatment with Lenalidomide (10mg/day on days 1 through 21 of repeated 28 days cycles) was started after red blood cell transfusions. After seven cycles a complete recovery of hemoglobin and platelet concentration was obtained (Hb 13 g/dl, PLT 180x10^9/L), while worsened leucocytosis needed supplementation with growth factor. Several authors have already reported the presence of del5q in MPN as well as in MDS with JAK2 V617F somatic mutation, furthermore some cases of ET and concurrent presence of del5q and JAK 2 mutation have been referred and finally Ingram and coll. have described six cases of MDS aborning del5q and JAK2 alteration. There is not yet an univocal evaluation of the prognostic significance concerning the association of JAK2 mutation and del5q in MDS or in MPN, in future probably new entities will be described. The use of Lenalidomide is a new more extensive application of molecular investigation. Here we underline the very good response of this patient to Lenalidomide therapy with normalization of thrombocytosis, previous resistant to the Hydroxyurea administration, and recovery of hemoglobin concentration with no further need of transfusion therapy.

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EXTRA-MEDULLARY LOCALIZATION IN HIGH RISK MYELODYSPLASTIC SYNDROMES: A NEW MODALITY OF RELAPSE AFTER THERAPY IN ELDERLY

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Background: extra-medullary localization in myelodysplastic syndromes (MDS) is a rare event but it may represent a pattern of relapse following allogeneic bone marrow transplantation in about 20% of acute myeloid leukemia (AML) (2). Features of relapse following hypomethylating agent therapy in high risk MDS included usually bone marrow leading to AML transformation. Purpose: we report 3 cases of extra-medullary localization following hypomethylating agent treatment in high risk MDS. Cases: Case 1 after 15 cycles of Decitabine, a 73 years old man developed mental impairment including confusion, somnolence and desorientation; Weight loss and disability with falls increased in few days. While blood analysis showed no features of relapse, cerebral fluid analysis revealed massive presence of blasts cells. Case 2 after 14 cycles of Azacitidine, a 72 years old man developed pulmonary symptoms (cough). Biopsy of a tumoral lung lesion was confirmed MDS relapse. Case 3 after 15 cycles of Azacitidine, a 80 years old woman developed diplopia due to ocular muscle impairment. MRI showed tumoral lesion of the right cavernous sinus. Bone marrow aspira-
tion showed no relapse of RAEB. Massive blasts infiltrate was detected in cerebral fluid analysis. Conclusions: extra-medullary localization in high risk MDS is a new modality of relapse following therapy. Diagnosis could be difficult, poor outcome is the rule. Role of hypomethylating agent in this issue should be studied.

References

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IRON CHELATION IN MYELODYSPLASTIC SYNDROMES: A DUAL CENTER EXPERIENCE
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Background. Iron overload is common in patients with myelodysplastic syndromes (MDS) who are treated with red blood cell (RBC) transfusions. Transfusion dependency is associated with leukemic progression and shorter survival. Guidelines recommend iron chelation therapy for management of iron overload, but little is known about chelation patterns in daily clinical practice. Aims. The objective of this dual centre study was to evaluate iron status and management in MDS patients, especially the utilisation of iron chelation therapy and chelation patterns. Method. A total of 77 patient records between January 2006 and December 2009 were analysed for this retrospective, cross-sectional, observational study. Results. Median age at diagnosis was 75 years. There were no statistically significant differences between chelated and nonchelated patients in terms of International Prognostic Scoring System score (IPSS score). Fifty-three patients had an IPSS score of low/intermediate-1 and hence eligible for assessment of iron chelation. Eighteen patients had received more than 25 RBC units in the past 12 months and therefore were eligible for iron chelation therapy. Medium serum ferritin was 1930 μg/L. Eleven patients (61%) did not receive any iron chelation therapy. Their median ferritin was 1930 μg/L. Reasons for not receiving iron chelation therapy were refusal (2), malignancy (1), parkinsonism (1), stroke (1) and age (98yrs) (1). No reasons were documented in five patients. Seven patients (39%) received iron chelation therapy with either deferoxamine (1), deferiprone followed by deferasirox (5) and deferiprone (5). Their median ferritin was 253 μg/L. None of the patients on deferoxine had a therapeutic response. Summary/Conclusions. Iron chelation therapy may be underutilized in transfusion-dependent MDS patients. This can be reduced by using a combination of clinical judgment, a serum ferritin level >1,000 μg/L and/or two or more RBC transfusions per month for the past year. The key reasons for not initiating iron chelation therapy were related to poor patient prognosis, patient age, and comorbidities. Deferoxnon was not an effective iron chelation agent in this study and warrants further study to demonstrate its effectiveness.

1282
THROMBOTIC EVENTS IN DEL(5Q)ASSOCIATED MYELODYSPLASTIC SYNDROMES.
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Background. Thrombocytosis is the hallmark of the 5q-syndrome but subsequent risk of thrombotic events remains unclear. JAK2 V617F mutation clones and del(5q) in myelodysplastic syndromes could be associated (1). Recent publications suggest that JAK2 and del(5q) arise in discordant clones (2). Whether if thrombotic events could be linked to JAK2 occurrence moreover than del(5q) is an unresolved question. Patients and methods: thrombotic events assessment in a cohort of myelodysplastic syndromes. Some patients have been previously described (3). Case reports: 15 patients, 12 cases of del(5q) and 3 cases without del(5q). Of the 12 cases of del(5q) 6 cases presented evidence of thrombotic events. All 15 cases presented a high risk disease score (IPSS score). Fifty-three patients had an IPSS score of low/intermediate-1 and hence eligible for assessment of iron chelation. Eighteen patients had received more than 25 RBC units in the past 12 months and therefore were eligible for iron chelation therapy. Medium serum ferritin was 1930 μg/L. Eleven patients (61%) did not receive any iron chelation therapy. Their median ferritin was 1930 μg/L. Reasons for not receiving iron chelation therapy were refusal (2), malignancy (1), parkinsonism (1), stroke (1) and age (98yrs) (1). No reasons were documented in five patients. Seven patients (39%) received iron chelation therapy with either deferoxamine (1), deferiprone followed by deferasirox (5) and deferiprone (5). Their median ferritin was 253 μg/L. None of the patients on deferoxine had a therapeutic response. Summary/Conclusions. Iron chelation therapy may be underutilized in transfusion-dependent MDS patients. This can be reduced by using a combination of clinical judgment, a serum ferritin level >1,000 μg/L and/or two or more RBC transfusions per month for the past year. The key reasons for not initiating iron chelation therapy were related to poor patient prognosis, patient age, and comorbidities. Deferoxnon was not an effective iron chelation agent in this study and warrants further study to demonstrate its effectiveness.

JAK2 mutation in 2 cases (1 DVT and 1 intra-abdominal thrombosis), post-surgery period in 1 case and lenalidomide treatment initiation in 2 cases. In the two remaining patients no risk factor was found. During the same period 1 case of DVT and 1 ischemic stroke occurred in non del(5q) MDS. Conclusions. Spontaneous risk of thrombosis in del(5q) seems to be higher than in overall MDS population. Some additional risk factors have to be evaluated as JAK2 mutation especially if intra-abdominal thrombosis was observed. Thrombotic risk prevention could be discussed in the field of del(5q) treatment.

References

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PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) IN THE CANARY ISLANDS: DESCRIPTION OF NINE CASES
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PNH is a very rare clonal stem cell disorder due to an acquired mutation of PI-G-A gene which causes deficiency of glycoprotein Iphosphatidyl nositol and several proteins linked to it. Some of these proteins play a central role in protecting cells from the lityc activity of complement, therefore rendering blood cells highly sensitive to complement. PNH is a chronic disease with predisposition for thrombosis and variable degrees of bone marrow failure. We present the data of 9 patients diagnosed and treated in our community Material and methods. Median age at diagnosis was 48y (33-70), Male/female ratio was 2:1. Blood counts median were Hb =91gr/L, Platelets=158x10^9/L and WBC=4.1x10^9/L. Medium LDH=1855μUL (n<247), bilirubin 1,7 mg/dl (N<1.4) and creatinine 1.15mg/dl (N<1.4). All patients, except one with aplastic anaemia, had classical PNH. 7 of 9 had moderate to severe quality of life impairment that interfered with laboral and social life due to chronic invalidating fatigue or abdominal pain. 4 cases had one thrombotic episode in different territories: one right leg deep vein thrombosis, one portal vein, one suprahepatic veins and one CNS vein stroke. All patients are since then on oral anticoagulation. 4 cases had chronic renal damage and 2 others experienced acute renal failure during a crisis. Diagnostic was performed by flow citometry analyzing CD16, and CD55 expression in granulocytes and CD55 and CD59 in red cells. 8 of 9 cases had a granulocyte size clone over 50%. Bone marrow cellularity was increased except in the case with aplastic marrow. No cytogenetic abnormalities were detected in any case. All patients received folic acid suplementation and iron if deficiency was detected. 5 cases were transfusion dependent regularly and 2 were occasionally transfused during an acute attack. Due to the cost of Eculizumab health authorities in our community encouraged the design of a therapeutic guide by hematologists for PNH patients. In summary according to our guide, therapy with eculizumab was indicated in adult patients if they were transfusion dependent or poor quality of life and had a size clone type III>10% with overt hemolysis (LDH>1.5 over normal range) and preserved marrow function (platelets>30,000). Chronic renal damage (not justified by other causes) or thrombotic episode were by themselves indication for therapy. 7 patients started eculizumab after meningococceal vaccination last year. No adverse reaction was seen during infusion. 5 of the patients became transfusion independent and the rest had a dramatic reduction in transfusion requirements. 1 patient with chronic renal failure normalized serum creatinine (2.3mg/dl to 1.3) In one patient with breakthrough haemolysis the dose was increased to 1200mg every 2 weeks with good response. No Coombs positive extravascular haemolysis has been observed so far. All patients wether transfusion dependent or not experienced a marked improvement in quality of life Conclusions The prevalence of PNH in our community is 0.43 per 100,000 inhabitants. Clinical data does not differ essentially from other series. Although follow up in patients with eculizumab in our community is too short tolerance end response seems to be excellent. Due to the cost of eculizumab clinical guidelines could help in decision making.
DEMOGRAPHIC AND SURVIVAL DATA OF INCIDENT CASES OF MDS/CMML IN MANITOBA DURING 2006-2007: DIFFICULTIES IN RETROSPECTIVE PROGNOSTICATION

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Background. With the availability of newer, but more expensive therapies for MDS, demographics and prognostic scoring are increasingly important to allocate resources for health planning. We have earlier reported on the Incidence of MDS/CMML in Manitoba during the years 2006-2007 (Kumar et al Blood 2009; 114: Abstract245). This cohort provided us with an opportunity to study prognostic characteristics of this cohort. Aims. To study the demographic and prognostic characteristics of MDS patients in Manitoba for health care planning. Methods. After obtaining ethics sanction, the clinical records of all incident cases of MDS/CMML diagnosed during a two year period (2006 and 2007) were retrospectively studied to categorize the subtype according to the WHO classification and determine the prognostic features. The Manitoba Cancer Registry was used to study survival data. Kaplan-Meier (KM) curves were used for estimates of median overall survival. Log rank test was used to compare KM estimates between groups. All analyses were conducted using SAS version 9.1. Last date of follow up was Apr 30, 2010.

Results. The newly diagnosed cases of MDS/CMML in the study period of two years consisted of 45 males and 37 females with a median age of 73 yr (range 45-90). Nine patients were <60 yr (11%). The following subtype categories and number of patients were identified: RA- 9; RARS- 11; RCMD-22; RCMD-RS- 2; RAEB1- 5; RAEB2- 7; MDS del(5q) -1; MDS-U- 12; MDS/CMML -7 and MDS/MFD- 4. Bone marrow cytogenetics were available in only 17 patients and could be classified in the following categories: Good risk -10, Intermediate -2, Poor -5. IPSS Score was determined in 17 Pts - Low risk: 5, Int-1: 3; Int-2: 7; High risk: 2. Blood counts at initial diagnosis were available in 75 patients with the following median values: Hb 91g/L (range 55 -140); Platelets 124x109/L with ANC < 0.5x109/L in 11%. There was little data on ferritin values and the WPSS scoring was not possible in 65 Pts (81%). The median OS was 14.4 months (95% CI 9.96-25.22). Median OS for females was 12.04 months and 18.40 months for males (difference not significant p= 0.45). Conclusions. This retrospective analysis showed a poor OS in the cohort. It could not accurately determine the prognostic group of most patients due to inadequate investigations or records. For population based prognostic scoring, there is a need to create MDS Registries and collect data prospectively.

APLASTIC ANEMIA IN CHILDHOOD: 8-YEARS EXPERIENCE AT A SINGLE INSTITUTION

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Background. Aplastic anemia (AA) is a rare acquired or inherited syndrome of bone marrow failure and early stem cell deficiency characterized by peripheral pancytopenia, bone marrow hypoplasia, and mild macrocytosis due to stress erythropoiesis. Chemical exposure, medicines, viral infections and immunodeficiencies are the aetiological factors responsible. Immunosuppressive therapy (IST) and allogeneic bone marrow transplantation (BMT) have improved the outcome of AA in childhood, though the best treatment options remain to be established. Aims: Diagnostic evaluation, treatment, short and long term follow up and outcome of children with AA who were hospitalized at a single Pediatric Hematology Oncology Unit. Materials and Methods. Retrospective analysis of children with the diagnosis of moderate or severe AA in the last 8 years (aetiological factors, treatment response, and overall survival rates). Results: Seven children were diagnosed with AA with mean age 9.2 years (±1.6), of which five (71%) were girls. The main initial diagnosis was made in March 2005, following flow cytometry analysis showing loss of CD55/CD59 expression on granulocytes, monocytes, and lymphocytes (Table 1). In June 2005 he presented with fatigue, coke-colored urine, decreased hemoglobin, and a positive Ham’s test. With no related donor, 3 compatible umbilical cord blood units were located in New York. He received treatment with fludarabine, melphalan, and thymoglobulin with prophylactic tacrolimus and serolimus. He underwent BMT in September 2007. Post- BMT, he required almost 5 months hospitalization for severe anemia requiring RBC transfusions, infections with undetermined focus, and furunculosis. In March 2008, secondary bone marrow failure was detected; he experienced a severe hemolytic crisis with anemia requiring multiple RBC transfusions, leukopenia, indirect hyperbilirubinemia, and high corpuscular volume. The patient described strong abdominal pain, back pain, severe headaches, fatigue at rest, and hematuria. Flow cytometry in July 2008 showed loss of CD55/CD59, indicating reappearance of PNH (Table 1). Due to severe symptomsology, he was placed on eculizumab therapy in July 2009, and experienced sustained hematologic response. He continues therapy with eculizumab, cyclosporine, folic acid, and ferrous fumarate without complications. Conclusions. This is a unique case detailing the reappearance and expansion of PNH clones following BMT. Although BMT is considered a potentially curative option in PNH, it is associated with a high risk of mortality and morbidities, and may not be curative in all patients. In light of the improved survival of PNH patients on eculizumab from clinical trials and single-center studies, this case highlights the potential benefits of eculizumab and reinforces the complexity of considering potentially dangerous treatment options such as BMT in PNH patients.
B19 infection in 3 children, recent HCV infection in 1 child). One child suffered from inherited AA (Fanconi anemia). Of all the children, two received only supportive treatment (28.6%), while the remaining five (71.4%) received combination therapy with cyclosporin A and antithymocyte globulin (ATG) and G-CSF. The response rate to treatment was 28.5%. Mean follow up was 5.3 years. Four out of seven children (57%) underwent finally bone marrow transplantation (78% response). The overall survival rate was 57.1%. Conclusions. The course of the disease to recovery is variable but is similar to that reported in the international literature. The role of etiologic factors and the best treatment options in childhood AA is not fully disambiguating and needs further investigation.

**References**

1. Calado RT, Telomerese and marrow failure. Hematology Am Soc Hema-


**Conclusions**

Dyskeratosis congenita (DC) is a genetic syndrome including usually abnormal cutaneous pigmentation, nail dystrophy and mucosal leukoplasia. In addition, an increased risk of neoplasia, myeloid hemophagocytosis, organ fibrosis (lung, liver) and dysimmunity is described. Patient survival is strongly impaired by bone marrow failure. Pleomorphic presentation of the disease leads to frequent misdiagnosis. We report the case of a 53 years old man with bone marrow hypoplasia and severe lung fibrosis. The diagnosis of DC was performed and a new TERT (Telomerase reverse transcriptase) mutation was described. Familial history of cirrhosis (brother) and association between bone marrow failure and lung fibrosis were significant parameters to assess the diagnosis. Conclusion: New modality of transmission of DC were recently described. Recessive X-linked (DKC1 mutation) and recessive autosomal form (TERC mutation) were first described. More recently, mutations with regard to telomerase complex’s proteins (TERT, NOP10, NHP2) and its steady proteins were reported. These new mutations allow us to explain different clinical pathways and transmission’s forms of the disease.

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1. Calado RT, Telomerese and marrow failure. Hematology Am Soc Hema-


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**DYSKERATOSIS CONGENITA A POTENTIAL CAUSE OF BONE MARROW FAILURE**

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**Background.** Dyskeratosis congenita (DC) is a genetic syndrome including usually abnormal cutaneous pigmentation, nail dystrophy and mucosal leukoplasia. In addition, an increased risk of neoplasia, myeloid hemophagocytosis, organ fibrosis (lung, liver) and dysimmunity is described. Patient survival is strongly impaired by bone marrow failure. Pleomorphic presentation of the disease leads to frequent misdiagnosis. We report the case of a 53 years old man with bone marrow hypoplasia and severe lung fibrosis. The diagnosis of DC was performed and a new TERT (Telomerase reverse transcriptase) mutation was described. Familial history of cirrhosis (brother) and association between bone marrow failure and lung fibrosis were significant parameters to assess the diagnosis. Conclusion: New modality of transmission of DC were recently described. Recessive X-linked (DKC1 mutation) and recessive autosomal form (TERC mutation) were first described. More recently, mutations with regard to telomerase complex’s proteins (TERT, NOP10, NHP2) and its steady proteins were reported. These new mutations allow us to explain different clinical pathways and transmission’s forms of the disease.

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1287a

**IRON CHELATION THERAPY WITH DEFERASIROX IN TRANSFUSION DEPENDENT MYELODYSPLASTIC SYNDROME PATIENTS**

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**Background.** The majority of low-intermediate risk patients (pts) with MDS require red blood transfusions, which can result in iron overload and its clinical sequelae. The recent development of a safe and efficient once daily oral iron chelator (Deferasirox, Exjade) made possible regular chelation therapy in transfusion dependent MDS patients. Methods. Fifty-nine transfusion dependent IPSS low-intermediate1 risk MDS patients were studied. Key inclusion criteria were requiring transfusion for anemia; median age was 67 years (range 25-84); 28 were IPSS low risk and 31 Intermediate1; duration of transfusion dependency before treatment was 24 months (12-36) corresponding to 58 (22-72) packed red blood cells transfusions received. Baseline serum ferritin was 2000 ng/ml (1471-5800). Mean duration of transfusions 3.5 years (1-5) MDS therapy included hydroxurea, anagrelide and growth factors. Patients started treatment with the standard 20 mg/kg Deferasirox dose but dose adjustments on clinical indications were done. Results. Over 12 months the mean dose of Deferasirox was 20mg/kg/day, and the mean transfusion rate was 3.4 units/months. The reduction in ferritin level was achieved after 5 months of treatment. Hematological improvement by IWG2000 criteria was achieved in 7 pts (11.8%); erythropoietic response in 5; platelet in 1 and neutrophil in 1. Conclusions. In these MDS iron overloaded pts, Deferasirox was generally well tolerated. Serum ferritin behavior confirms Deferasirox efficacy. The serum ferritin reduction was more evident in the more heavily overloaded population indicating successful iron depletion in this group of patients as clinically requested.

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1288

**CYCLOSPORINE A MONOTHERAPY IN CHILDREN WITH SEVERE APLASTIC ANEMIA: SINGLE CENTER EGYPTIAN EXPERIENCE**

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**Background.** Immune suppression (IS) therapy has provided an opportun-
yty of cure or improvement in the aplastic anemia patients who are not feasible candidates for bone marrow transplantation. Although the combination of cyclosporine A (CSA) and antithymocyte globulin (ATG) is superior to either agent alone, CSA monotherapy is an easily available, safe and cheap (IST) option. These advantages are particularly valuable in developing countries where ATG is frequently not available. Methods. In the Hematology Department at Children’s Hospital Ain Shams University, 35 patients (24 males and 11 females), with a median age of 3.5 years with severe aplastic anemia (SAA) were prospectively identified and managed with CSA monotherapy at a dose of 6-10 mg/kg/day (divided every 12 hrs) during the period between January 2006 and January 2011. Seven patients (20%) expressed PNH-like clones at diagnosis, 3 patients (8.5%) had non-A, non-B, non-C hepatitis 3-6 weeks before presentation, one patient gave history suggestive of drug toxicity, 5 patients had a preceding upper respiratory tract infection, and in the remaining patients, no possible cause was identified (idiopathic). Results. 30 eligible patients giving parental consent were treated with cyclosporine for at least 6 months. After 6 months of therapy, 4 patients (12.5%) achieved complete remission (CR); 9 patients (30%) achieved partial remission (PR). After one year of therapy 10 patients (33.3%) achieved CR and 11 (36.7%) remained in PR. Two patients (1.5%) lost follow up and 2 patients (1.5%) died of serious septic and bleeding events before the fourth month. Discontinuation of CSA before the sixth month occurred in one patient for serious neurotoxicity, otherwise, other side effects were modest and easily monitored. Short term steroid therapy was used in frequently transfused alloimmunized children. Hospital admission was more frequent during the first 3 months of IST and was mainly for febrile neutropenia and serious infection. Younger age, shorter interval between diagnosis and treatment, lower monthly requirement of platelets transfusion, higher hemoglobin levels before IST therapy, and, higher bone marrow cellularity were positively associated with response. Conclusions. In developing countries where facilities are modest and most patients cannot afford adequate treatment of SAA, CSA monotherapy can provide an available easily monitored immunosuppressive tool for patients with SAA.
(Kappa statistic 0.25-0.23) while on Pelger abnormality and anisopoikilocytosis moderate agreement was reached (Kappa 0.45-0.46). Conclusions. Evaluation of blood films in a cohort study is difficult as transport times to the laboratory adversely affect film quality. Interobserver concordance on early blood film features is poor - limiting the value of film analysis for early myelodysplastic features. Film analysis for features of haematocytic deficiency - the differential for myelodysplastic syndrome was also unsatisfactory.

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LOW PERCENTAGE OF PERIPHERAL BLOOD DENDRITIC CELLS IN PATIENTS WITH MULTIPLE MYELOMA CORRELATES NEGATIVELY WITH INTERLEUKIN-6 AND BETA-2-MICROGLOBULIN LEVELS
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Several studies demonstrate the presence of quantitative and functional abnormalities in the dendritic cell subsets in patients with multiple myeloma (MM). The inhibitory effect of IL-6, TGF-β, IL-10 and beta-2-microglobulin is highly suspected. The aim of the study was to evaluate the myeloid and plasmacytoid dendritic cells (MDC and PDC) in newly diagnosed patients with MM in correlation with various biological markers. Thirty patients with newly diagnosed MM were included in the study. All the laboratory parameters were obtained at time of diagnosis. The color flow cytometry with ILT3/lin/CD11c was used for the detection of the two peripheral blood DC subsets. The plasma levels of IL6 were detected by ELISA (standard curve range: 2-200 pg/ml). The median age of patients was 61.5 years (36-89). The mean M-protein concentration was 46.5±16.2 g/l. IgG kappa was detected in 15 patients, IgG lambda in 4, IgA kappa in 7, IgA lambda in 8. The mean level of beta-2-microglobulin was 7.0±5.7 mg/l (1.82 - 22.49 mg/l); beta-2-microglobulin was used to determine the stage according to the ISS. The mean level of IL6 was 27, 73±21.47 pg/ml 4.6-72.5 pg/ml. The percentage of MDC and PDC were significantly lower in the patients with MM in comparison to healthy subjects (0.08%±0.09% vs 0.21%±0.02% and 0.04%±0.03% vs 0.16%±0.01%, respectively). A statistically significant difference was found between the percentage of MDC and PDC in the different stages. There was a negative correlation between MDC and PDC and the levels of beta-2-microglobulin (p=0.02 and p=0.02), as well as between MDC and the IL6 levels (p=0.04). No correlation was found between MDC, PDC, levels of M-protein and the type of paraprotein. Our results demonstrate the relationship between peripheral blood DC, IL6 and beta-2-microglobulin and confirm the published data for the inhibitory effect of the two factors on DC differentiation and maturation in vitro. The monitoring of IL6 and beta-2-microglobulin may have clinical implications as a predictor of the immune system status as well as for the yield of harvested DCs for vaccination.

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ASSOCIATION STUDIES OF FUNCTIONAL ICOS GENE POLYMORPHISMS WITH MULTIPLE MYELOMA IN THE POLISH POPULATION
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Background. Inducible costimulatory molecule (ICOS) which is expressed on the T-cell surface after activation enhances all the basic T-cell responses to a foreign antigen, namely proliferation, secretion of lymphokines, the up-regulation of molecules that mediate cell-cell interaction, and effective help for antibody secretion by B cells. Aims. The study was undertaken to evaluate the association between three ICOS polymorphisms (which were recently described as functional ones) and susceptibility to multiple myeloma (MM) in a Polish population. Methods: A case-control study of 454 individuals including 201 MM patients was conducted on polymorphisms in the ICOS gene. Genotyping of the polymorphisms ICOSIV1+173T>C, ICOSC.1624C>T, and ICOSC.602A>C and ICOSC.1564 T>C was done using allele discrimination methods with the TaqMan SNP Genotyping Assay. Results: We noted the frequencies of alleles ICOSIV1+173T>C [T], ICOSC.602A>C [A], and ICOSC.1564 T>C [T] were higher in MM patients than in healthy controls, but differences did not reach statistical significance (0.91 vs. 0.88, p=0.17; 0.82 vs. 0.79, p=0.19 and 0.83 vs. 0.79, p=0.2, respectively). The distribution of alleles and genotypes for ICOSC.1624C>T polymorphism was similar in both groups. Conclusions. The result of present study does not strictly confirm the association of investigated polymorphisms with susceptibility to MM due to small size of studied group, but warrant further investigation through replication studies.
the treatment with statistical significant difference at month 3rd (1.18±0.46 µg/mL) and 6th (1.17±0.52 µg/mL), respectively p=0.04 and p=0.05. Venous thrombosis (DVT) occurred at the time of treatment in eight (12.5%) of MM patients. The study revealed tendency of a modulating effect of protein Z on the DVT in the entire group of patients, as well in the group with FZ concentration below 5th percentile, but not with significant correlation p=0.13 and p=0.08 respectively. Conclusions. This reduction to the level below 5th percentile during IMIDS containing therapy may be an additional puzzle in explanation of the increased rate of thrombosis in MM patient treated with thalidomide.

**1293**

A NEW INDICATION FOR AN OLD DRUG: THERAPEUTIC POTENTIAL OF PROPRANOLOL ON HUMAN MULTIPLE MYELOMA CELLS

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Background. Propranolol, a nonselective β-adrenergic receptor blocker, has been used for the treatment of the patients with hypertension for more than 50 years. Propranolol is also used in the treatment of angina pectoris, anxiety, tachycardia, arrhythmia, tremor, migraine, panic attack, and thyrotoxicosis. There are several in vitro and in vivo evidences that β-adrenergic receptor antagonists inhibit proliferation and angiogenesis, decrease tumor metastasis, and increase apoptosis in breast, stomach, skin, and colon cancers. Multiple myeloma is the second most common hematological malignancy. Despite of the high dose chemotherapy and other treatment approaches, there is no efficient curative approach yet. Aims. In this study, we aimed to investigate the cytotoxic and apoptotic effects of propranolol, the nonselective β-adrenergic receptor blocker, on U266 human multiple myeloma cells. Methods. Time-dependent proliferation of U266 cells exposed to increasing concentrations of propranolol was determined by MTT cell proliferation assay. Apoptotic effects of propranolol on human U266 multiple myeloma cells were examined by analyzing the changes in caspase-3 enzyme activity, mitochondrial membrane potential, and also the localization of phosphatidylserine in the plasma membrane. For this purpose, caspase-3 colorimetric assay kit, JC-1 mitochondrial membrane potential detection kit and Annexin V-FITC apoptosis detection kit were used, respectively. Results. IC50 values (drug concentrations that inhibit the proliferation of cells by 50%) of propranolol in U266 cells were calculated as 141±100, 75 µM after 24, 48, and 72 hour propranolol exposure, respectively. Incubation of U266 cells with 50-, and 100 µM propranolol for 48 or 72 hours resulted in 10 and 18% or 46 and 50% increases in caspase-3 enzyme activity, respectively. The same concentrations of propranolol resulted in 8 and 55% increases in loss of mitochondrial membrane potential after 48 hours, and 75 and 77% increases in loss of mitochondrial membrane potential after 72 hours, respectively, as compared to untreated control group. To confirm these data we detected apoptotic cells by examining the localization of phosphatidylserine. The results revealed that propranolol induced apoptosis significantly in a time- and dose-dependent fashion in multiple myeloma cells. Summary/Conclusions. These results revealed that propranolol has antiproliferative and apoptotic effects on U266 human multiple myeloma cells. Being supported by in vivo analyses, propranolol can be a good and economical way to treat multiple myeloma patients.

**1295**

DETERMINATION OF CYTOTOXIC EFFECTS OF GOSSYPOL ON MULTIPLE MYELOMA CELLS

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Background. In multiple myeloma, plasma cells reproduce in an uncontrolled manner due to a number of molecular changes such as chromosomal translocations, point mutations and oncogene activation. Then, multiple myeloma cells produce monoclonal type of immunoglobulin. Multiple myeloma is diagnosed with the detection of more than 30% plasma cells in bone marrow. Furthermore, the cancerous cells accumulate in the bones and bone marrow. In the treatment of multiple myeloma, different agents have been administered as standard for many years. Although some novel agents increased the quality of life and survival times of patients, cure is still not possible and serious side effects of the agents still remain. Therefore, the authors investigated to find out novel agents are very important. Gossypol is a natural phenol derived from the cotton plant. Gossypol can induce apoptosis through downregulating Bcl-2 and upregulating Bax. It also inhibits protein kinase D. Gossypol has antimarial, antiviral activities in addition to its anticancer potential. Aims. In this study, we aimed to identify the cytotoxic and apoptotic effects of gossypol on multiple myeloma cells. Methods. Multiple Myeloma cells lines, ARH77, were cultured in RPMI1640 medium containing 10% FBS and 1% penicillin-streptomycin. The cytotoxic effects of gossypol on ARH77 cells were conducted via XTT cell proliferation assay. Cytotoxic effects of gossypol on ARH77 cells were conducted via XTT cell proliferation assay. Results. The XTT data showed that there were dose-dependent decreases in proliferation of ARH-77 cells. There were 29, 45 and 61% decreases in cell proliferation in response to 10, 20 and 50 µM gossypol, respectively compared to untreated control group. Apoptotic effects of gossypol on ARH-77 cells were evaluated by the determination of caspase-3 and mitochondrial membrane potential in ARH-77 cells, respectively. Results. The results of this study showed that sunitinib can be an alternative treatment approach for multiple myeloma. These studies should be confirmed further by in vivo analyses.

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APOTOTIC EFFECTS OF SUNITINIB ON MULTIPLE MYELOMA CELLS

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Background. Multiple myeloma is the second most comutatable myeloma hematological malignancy characterized by abnormal increases in the number of plasma cells. The direct interaction of the multiple myeloma cells with the other cells within the microenvironment has a vital importance for the progression of the disease since these interactions triggers the release of some cytokines and growth factors, critical for signaling pathways for multiple myeloma cells and for the cells in the microenvironment. Different therapeutic agents and treatment protocols were used for the treatment of multiple myeloma and some of them could increase survival time and quality life of the patients. But, despite these successes, multiple myeloma still remains to be an uncurable disease. Sunitinib is a multi-targeted receptor tyrosine kinase inhibitor that repress the activity of many receptors involved in important signaling pathways that regulate cell growth, proliferation and apoptosis. Aims. In this study, we aimed to decipher the cytotoxic and apoptotic effects of sunitinib on multiple myeloma cells and determine the roles of caspase-3 and mitochondrial membrane potential in sunitinib-induced apoptosis. Methods. Antiproliferative effect of sunitinib on U266 multiple myeloma cells was determined by XTT cell proliferation assay. Apoptotic effects of sunitinib on U266 cells were evaluated by the determination of changes in caspase-3 enzyme activity, mitochondrial membrane potential and Annexin V assays. These analyses were conducted by using caspase-3 colorimetric enzyme activity assay, JC-1 mitochondrial membrane potential detection kit and Annexin V-FITC kit, respectively. Results. IC50 value of sunitinib, the drug concentration which inhibits cell proliferation by 50% as compared to untreated control group, was calculated from cell proliferation plots single type to be 4 µM. There were 7, 17 and 20% increases in loss of mitochondrial membrane potential in response to 1, 5 and 10 µM sunitinib, respectively, compared to untreated control. The same concentrations of sunitinib increased caspase-3 enzyme activity by 10, 50 and 160%, respectively comparing to control group. In order to confirm these results, apoptotic deaths were detected by AnnexinV and results revealed that 1, 5, 10 µM concentrations of sunitinib increased 9, 55 and 40% of U266 cell population, respectively. Summary/Conclusions. The results of this study showed that sunitinib can be an alternative treatment approach for multiple myeloma. These studies should be confirmed further by in vivo analyses.
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EFFECTS OF BRUCINE ON METABOLISM OF OSTEOCLAST AND OSTEOLAST IN MULTIPLE MYELOMA

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This study was aimed to explore the influence of brucine on the early differentiation of osteoclast and the metabolic pathway of osteoclast in multiple myeloma (MM) and to compare the effects between brucine and bortezomib on MM. MTT method was used to determine IC50 of brucine and bortezomib on the MM cell line U266. The supernatant of cultured U266 cell line was added to the culture system for inducing the differentiation of osteoclast cell line MG63-E1. After aseptic assay, RT-PCR was used to determine the mRNA levels of alkaline phosphatase (ALP), osteocalcin (OC), osteoprotegerin (OPG) and osteoprotegerin ligand (RANKL). As a result, the median inhibitory concentration (IC50) of bortezomib on U266 cell line for 48h was 22.4 nmol/L and that of strychnine was 0.16 nmol/L. The mRNA levels of ALP, OC and OPG in osteoclast co-intervened by brucine combined with the supernatant of MM cells (p<0.05). The degree of increasing or reducing was larger than the level of control group intervened only by bortezomib (P<0.05). The above-mentioned results indicated that the therapeutic effects of brucine on osteoclast might be carried out through the regulation of osteoclast by osteoblast, and the experiment confirmed that the osteopetrotic effects of brucine on MM was superior to that of bortezomib.

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EVALUATION OF T REGULATORY AND TH17 CELLS RELATED GENES EXPRESSION IN BONE MARROW ASPIRATES OF SOLITARY PLASMOCITOMAS AND MULTIPLE MYELOMA PATIENTS

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Introduction. Underlying biological mechanisms of multiple myeloma (MM) involve a series of genetic alterations and changes in the bone marrow microenvironment, favoring the growth of the tumor and the failure of the immune system in controlling it. T regulatory (Treg) cells play an important role in the maintenance of self-tolerance and modulation of overall immune responses against infections and tumor cells. Th17 cells have a critical function in eliminating extracellular pathogens and tumor cells. The balance between Treg and Th17 cells may be essential for maintaining homeostasis of anti-tumor immunity. In this scenario, the aim of this study was to characterize the expression of Treg and Th17-related genes in bone marrow (BM) aspirates of MM and solitary plasmocytomas (SP) to evaluate their potential as therapeutic targets in these diseases.

Material and Methods. Expression of Foxp3 and ROR-γt genes, respectively associated with Treg and Th17 subpopulations, were determined by quantitative real-time PCR (RQ-PCR) in BM aspirates of 37 newly diagnosed MM patients, 04 newly diagnosed SP and 05 healthy controls (allogeneic transplant donors). Genes were considered overexpressed when RQ-PCR results showed values at least 2 times higher in cases than in normal samples.

Results. Foxp3 was overexpressed in 72% of MM cases. A 5.89-fold increase in Foxp3 expression was observed in MM patients compared to controls (p=0.0476, Mann-Whitney test). The other hand, MM patients and controls showed equal levels of ROR-γt expression and the difference between groups was not significant. Also, SP BM aspirates showed Foxp3 and ROR-γt levels similar to controls. Conclusions: Over-expression of Foxp3 in MM cases suggests an accumulation of immunosuppressive Tregs in the tumor environment and/or an immediate involvement of this gene in the development and progression of myeloma. Therapeutic approaches that specifically target Foxp3-expressing Tregs may provide more focused treatment strategies for MM.

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POINT MUTATIONS IN KINASE AND PSEUDOKINASE DOMAINS OF JAK1 GENE DO NOT SEEM TO BE RESPONSIBLE FOR ACTIVATION OF JAK/STAT PATHWAY IN MULTIPLE MYELOMA

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Background. JAK/STAT pathway, which can be persistently activated in multiple myeloma (MM) patients due to constant stimulation by IL-6, was recently explored by Burger et al (2009) as potential therapeutic target in this still incurable disease: Janus Kinase (JAK) inhibitor INCB20 presented antiproliferative and apoptotic effects on human myeloma cells in vitro and in vivo. Anns. To search for point mutations in JAK1 gene kinase and pseudokinase domains in an attempt to define any critical and recurrent alteration that could be used as therapeutic target for MM. Patients and Methods. We obtained RNA from purified CD138-positive cells from MM bone marrow samples using microbeads conjugated to monoclonal anti-human CD138 (sudecan-I) by the MACS methodology - Magnetic Cell Sorting of Human Cells (Miltenyi Biotec, Bergisch Gladbach, Germany) from 21 newly diagnosed patients MM, four healthy controls (one peripheral blood and three reactive tonsils) and four MM cell lines (U266, Sko-007, SKMM-2, RPMI). After amplification of of JAK1 pseudokinase (exons 12-18) and kinase (exons 19-24) domains in cDNA samples, we performed automatic sequencing of fragments using forward and reverse primers. Results: 15 of the 21 (71%) MM cases showed at least one polymorphism, all synonymous SNPs, being 12/15 in codon 733 (CCA>CCG), one in codon 683 (AGC>AGT) and 1 in codon 1032 (AAG>AAA). The mRNA levels of ALP, OC and OPG in osteoblast co-intervened by brucine combined with the supernatant of MM cells (p<0.05). The above-mentioned results indicated that the therapeutic effects of brucine on MM was superior to that of bortezomib.

Conclusions. Mutations in kinase and pseudokinase domains of JAK1 gene do not seem to be important for activation of JAK/STAT pathway in multiple myeloma and other underlying mechanisms, besides IL-6 stimulation, must be investigated.

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THE INFLUENCE OF CYTOKINE INTERLEUKIN-16 IN MULTIPLE MYELOMA

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Multiple myeloma is a malignancy characterized by the expansion of a plasma cell clone that localizes to the human bone marrow. Myeloma cells and a bone marrow stroma cells both produce soluble factors promoting the survival and progression of multiple myeloma. Interleukin-(IL)-16 is involved in regulating migration and proliferation of normal leukocytes, however, it has been unclear whether IL-16 also plays a role in the pathophysiology of human cancers. We found IL-16 to be strongly overexpressed in the bone marrow of myeloma patients. Myeloma cell lines as well as primary tumor cells from myeloma patients constitutively expressed IL-16 and its receptors CD4 and/or CD9 and spontaneously secreted soluble IL-16. Silencing of IL-16 had an anti-proliferative effect on the tumor cells which could be reversed by the addition of the C-terminal fragment of soluble IL-16. Most importantly, the application of a monoclonal antibody directed against IL-16 had a strong growth-inhibiting influence on myeloma cells. These findings indicate that cytokine IL-16 is an important growth-promoting factor in multiple myeloma and a candidate for novel diagnostic, prognostic and therapeutic applications for this incurable human malignancy.
Therefore, it has been hypothesized that loss of Rb gene might be associated with large segments or the entire long arm involving the retinoblastoma (Rb) gene. In myeloma, Rb gene is believed to downregulate interleukin-6 (IL-6) which plays a central role in the pathogenesis of MM. Therefore, it has been hypothesized that loss of Rb gene might be associated with very high expression of IL-6 and subsequent IL-6 driven cell survival and bad prognosis.

**Materials and Methods.** Forty MM patients and 20 matched controls were included in this study. Interphase fluorescence in situ hybridization (FISH) analysis was performed using LSI 13q14-specific probe. Serum levels of IL-6 were determined by ELISA. All patients received conventional chemotherapy. Refractory patients received other therapeutic regimen of Thalidomide or Bortezomib. Results. Significant increase (p<0.001) of IL-6 production was recorded in patients with 13q deletion compared to patients with normal chromosome 13q status. These patients were also refractory to conventional chemotherapy but showed striking response to Thalidomide or Bortezomib. Conclusions. This study suggests that 13q deletion might be associated with increased expression of IL-6 and this could be a possible cause of the associated bad prognosis. In addition, the results also show the potential to improve responses in patients with refractory MM with the introduction of novel therapies.

**EVALUATION OF APOPTOTIC MARKERS IN MULTIPLE MYELOMA AND MONOCIONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE**

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Multiple myeloma (MM) is characterized by aberrant cellular responses to signals governing proliferation and apoptosis. Its first pathogenic step is a pre-malignant monoclonal gammopathy of undetermined significance (MGUS). With progression from MGUS to MM, complex genetic and/or epigenetic events occur in the malignant plasma cells (PCs) and in the bone marrow microenvironment that contribute to cell signalling pathways deregulation. The knowledge of these mechanisms may provide new markers for disease progression and new molecular targets for therapy. Apoptotic cell death may be triggered by several mechanisms involving death receptors and/or by mitochondria (extrinsic and intrinsic pathways). The intrinsic pathway centers on mitochondria as initiator of cell death. Multiple signals converge on mitochondria causing these organelles to release cytochrome c (cyt c) and other apoptogenic proteins into cytosol. The B-cell lymphoma-2 (Bcl-2) family proteins are the most prominent of the intrinsic pathway. In this family of some of them have pro-apoptotic properties, as Bax, Bad and Bid, while Bcl-2 and Bcl-Xl have an anti-apoptotic function. In contrast, the extrinsic apoptotic pathway relies on tumour necrosis factor (TNF) family death receptors for triggering apoptosis. TRAIL is a ligand that triggers and activates 4 receptors of TNF family, TRAIL-R1 and -R2, also known as death receptors, DR4 and DR5, and antagonistic or modulator receptors, TRAIL-R3 and -R4, also known as decoy receptors, DcR1 and DcR2. Many mechanisms may contribute to the complex effects of TRAIL, namely the differential expression of TRAIL-Rs or functional defects, like those related to cytoplasmatic inhibitors of apoptosis such as inhibitory apoptotic proteins as survivin. However, apoptosis deregulation during MGUS to MM progression is not yet clarified. AIMS: The aim of this study is to contribute to clarify the molecular mechanisms involved in MGUS and its evolution to MM, namely the involvement of apoptotic pathways. METHODS: For this purpose, we evaluated bone marrow PCs from 13 patients (6 MGUS and 7 MM). PCs were identified by flow cytometry by CD138 expression and malignant PCs were analyzed by gating the CD138+CD19- population. In malignant PCs we evaluated the expression of apoptotic proteins Bax, p53, Fas/Fasl, TNF/TNF-R1, TRAIL/TRAIL-R1-2, caspase 3 and the anti-apoptotic proteins of TRAIL-R3-4, survivin and Bcl-2. Some of the proteins involved in survival, namely the transcription factor, NF-kB were also performed. RESULTS: The preliminary results from our study show that MM patients have highest levels of malignant PCs compared to MGUS patients (95% vs 67%, p<0.05). On the other hand, MM PCs show a statistically significant increase in p53, caspase 3, survivin and NF-kB (p<0.05) and a statistically significant decrease in BAX/BCL-2 ratio (p<0.05). These results may be related to the survival and apoptosis resistance of MM PCs. CONCLUSIONS: Our preliminary data suggest that in the progression from MGUS to MM a deregulation in apoptosis and survival pathways occur. The clarification of these mechanisms may contribute to the identification of new prognostic molecular markers and selection of patients for molecular targeted therapies.
MULTIPLEXED PHOSPHOPROTEIN CELL SIGNALING ANALYSIS PREDICTS PATIENT-SPECIFIC THERAPEUTIC RESPONSE AND/OR OFF TARGET EFFECTS IN MULTIPLE MYELOMA

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Background Interaction of Multiple Myeloma (MM) cells with bone marrow microenvironment has a pathogenetic role in the disease and may confer tumor cell resistance to conventional therapies. A great need exists to understand the differential effect of treatment on myeloma as well as non-myeloma cells. We designed an ex vivo study to rapidly screen treatment combinations to predict treatment efficacy. Methods A panel of signal pathway inhibitor treatments was applied to fresh bone marrow aspirate samples from patients undergoing standard of care hematological work up for multiple myeloma. Bone marrow aspirates were immediately subdivided and treated ex vivo with a panel of molecular targeted inhibitors, including combinations thereof that target a particular pathway (autophagy, proteosome, angiogenesis, protein degradation, proliferation/survival, insulin response, and translation). Up to 48 different treatment conditions can be studied from 5ml aspirate. After overnight incubation the samples were placed in a preservative that suppresses fluctuations in kinase pathway proteins. CD138+ plasma cells were separated from CD138 negative cells via immunomagnetic sorting. Reverse phase protein microarrays were used to quantify 60 cell signaling proteins in both cell populations. Results At baseline, patients with active myeloma (untreated before or relapsed) exhibited an increased pro-survival signaling (TNFR1, Akt Ser473, Akt Thr308, B-Raf Ser445) and ligands (IL6, IL10, TNFα) in both CD138+ and bone marrow microenvironment cells. Only 1 case exhibited the activation of NFKBp65Ser536 in CD138+ plasma cells. Only in plasma cells, a subset of patients exhibited higher levels of Beclin1 and LC3B, suggesting a potential role of autophagy in MM aggressive phenotype. Untreated before and relapsed patients did not show a unique hyperactivated pathway, except in a patient treated with lenalidomide, relapsed after 6 months and subsequently bortezomib resistant. In this specific case, almost endpoints evaluated were pretty higher than the other subjects included in the study, except for autophagy markers and c-kit signaling. When we evaluated the response of this patient to the treatment ex-vivo with Lenalidomide 1uM and Bortezomib 100nM, alone or in combination, we were able to predict refractoriness, since the lack of protein profiling after treatment. Plasma cells isolated from patients previously exposed to Bortezomib did not differ from Lenalidomide-exposed ones. However, in vitro these drugs triggered pathways different. After 12 hours of exposure to lenalidomide we confirmed the Caspase8 upregulation, yet reported in literature, associated to increased levels of Bcl-2 Ser20 and reduced Caspase3 Asp175 and Caspase9 Asp330. Conversely, Bortezomib was not able to induce Caspase8 and Bcl-2 Ser70. Conclusion Taken together, our data confirm the value of the RPMMA assay to investigate improperly activated pathways converging on apoptosis triggering. The proposed assay conducted at beginning of the treatment is a valid mean to individualize therapy and improve off target effects in multiple myeloma.

THALIDOMIDE EFFECT ON ANGIogenesis AND IN CD57+ LYMPHOCYTE POPULATION OF MULTIPLE MYELOMA PATIENTS

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Background The thalidomide (thal) action has not been fully elucidated. This drug has been consolidated as a promising therapeutic option for multiple myeloma (MM) because of its antiangiogenic and immunomodulatory effect. The microvessel density (MVD) and the amount of bone marrow (BM) CD 57+ lymphocyte population from (MM) patients are correlated with disease activity. Aims To evaluate the bone marrow angiogenesis and CD57+ lymphocytes amount before and after 3 months on thal treatment. Materials and methods. We collected BM biopsies from 20 MM patients treated with Thal up front. The Thal dose was 200 daily. Samples were collected at diagnosis and after 3 months on that treatment. The angiogenesis was assessed by CD 34 staining that was estimated by microvesSEL-density (MVD) and cytotoxic lymphocytes / NK cells counting by CD 57+. Microscopy was performed by two independent blinded pathologists to the treatment time. Photographs of three areas of high vessel concentration were selected for CD 34 counting with 400X magnification. Analysis of cytotoxic lymphocytes / NK was performed by direct counting on three areas of high concentration for the CD57+ population. Results The MVD was significantly reduced after 3 months on thal treatment. VGPR=8, PR=7, MR=2, progression in 1 case and 2 were not analyzed. The reduction in MVD after 3 months with thal was significant (p=0.04). There was no significant difference in the amount of CD 57+ pre and post treatment. The response rate was not correlate with angiogeneses reduction or with the amount of CD57+ lymphocytes. Conclusions Evaluation after 3 months on thalidomide treatment showed a significant angiogenesis reduction. The number of patients may have been insufficient to evaluate the CD57+ lymphocytes population. Probably, the variations of lymphocytes occur in later treatment phases.

INVESTIGATION QUALITY OF LIFE IN MULTIPLE MYELOMA PATIENTS WITH ANEMIA RECEIVED RECOMBINANT HUMAN ERYTHROPOIETIN

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Background. Anemia in patients with Multiple Myeloma (MM) is a frequent symptom and can influence the efficacy of antitumor chemotherapy, survival rate and overall quality of life (QoL). Red blood cell (RBC) transfusions are commonly used to treat anemia, while recombinant Human Erythropoietin (rHuEPO) treatment has been shown to significantly increase hemoglobin (Hb) concentration, reduce the number of RBC transfusions and improve QoL in patients with chemotherapy induced anemia. Aims. This study was performed to find out the effectiveness Recombinant Human Erythropoietin in multiple myeloma patients with anemia and improving QoL. Methods. There was done prospective study to investigate the effectiveness of rhHuEPO (epoetin beta) on Hb concentration, RBC count and QoL in multiple myeloma patients with anemia (n=44). The median age of patients was 67 years (range 26-80). If Hb concentration was <8.0 g/dl, the patients were performed red blood transfusions before rhHuEPO treatment. Recombinant Human Erythropoietin beta was injected subcutaneously on 30.000 IU 1 times a week. Before start of rhHuEPO treatment all patients have been received two or more cycles of chemotherapy. The target Hb level was 12 g/dl. The positive response was estimated as increasing Hb concentration on 2.0 g/dl and so achieving target Hb level (12 g/dl). QoL was assessed by FACT-An questionnaire. Results. The initial Hb concentration was 9.02±1.19 g/dl and RBC count was 2.78±0.43x1012/L. 10 patients had Hb concentration less than 8.0 g/dl (5.6-7.9 g/dl) therefore they were transfused 2-5 units of red blood until Hb increased up to 8.0-9.0 g/dl. The period of rhHuEPO-therapy was from 4 to 16 weeks (mean 9.1±3.6 weeks and median follow-up of 9 weeks). During the study period in whole group, the Hb concentration and RBC count increased from baseline to 11.46±2.08 g/dl (p<0.01) and 3.56±0.79x1012/l (p<0.01), respectively. An Hb increase >2.0 g/dl was observed in 30 patients (68.2%), while a non-response was observed in 14 ones (31.8%). The patients with positive response significantly increased Hb from 9.05±1.06 to 12.41±1.11 g/dl (p<0.001) and RBC count increased from 2.78±0.41x1012/L to 3.78±0.51x1012/L (p<0.001). In this group the initial dose of rhHuEPO were reduced to 20.00 IU at 5 patients (16.7%) because of fast increasing their Hb (more than 2.0 g/dl during 4 weeks) to prevent arteral hypertension. In group non response patients there were not significant difference of Hb concentration and RBC count (from 9.05±1.06 to 9.20±1.91 g/dl) (p>0.02) and increased from 2.79±0.47x1012/L to 2.88±0.88x1012/L (p<0.02). Besides of out ten patients four ones (40%) had been continued to receive red blood transfusions. FACT-An demonstrated that rhHuEPO-therapy in group patients with positive response reduced such symptoms as: feeling fatigue, weakness all over, having trouble starting things because of tiredness, depression, drowsiness, giddiness, headaches, pain in thorax and dyspnea. Conclusions. The study has shown that rhHuEPO is effective at increasing Hb concentration, count of RBC and improving QoL in anemic patients with Multiple Myeloma.
The quantitation of the monoclonal immunoglobulins (Ig) and its fragments are used for the monitoring of Bence Jones myeloma (BJM) course and effect of therapy introduced. Bence Jones protein in urine (Ig free-light-chains (FLC)) is characteristic of light-chain multiple myeloma (LCMM). Relatively new laboratory tests in serum for the quantitation of kappa (κ) and lambda (λ) FLC and the calculation of the FLC ratio represents additional parameters of malignant plasmacytosis production. The aim of FLC examination was to evaluate significance of FLC and kappa/lambda ratio (k/λ ratio) as a prognostic factor for progression, remission and survival in BJM patients. The concentrations of Ig and FLC were measured by immunonephelometric method on a “SIEMENS” Dade BN II analyser. The concentrations of Ig and FLC were measured by immunonephelometric method on a “SIEMENS” Dade BN II analyser. In this examination 37 BJM patients were investigate during the period of last 7 years. Reference interval for k/λ ratio is (0.26–1.65). According to the ISS Model of stratification risk of disease progression, values <0.83 Ig > 50% represents high relative risk (R), <0.26 Ig > 1.65 represents low-intermediate risk and <0.125 Ig > 50% represents high-intermediate RR of disease progression. Results showed that in BJM group 19/37 (51.4%) patients had high RR; 7/37 (18.9%) had low-intermediate RR, while 8/37 (21.6%) patients had high-intermediate RR. Also, 3/37 (8.1%) patients had normal k/λ ratio, low RR and good prognosis. Nine patients was died during the period of 24 - 86 months. About 28% patients which have lowered FLC values more than 50% under therapy, achieved the disease remission in group with BJM myeloma patients. Abnormal FLC ratio in group of BJM patients, could be an independent risk factor of progression and a worse disease prognosis. Serum assays could replace Bence Jones protein urine tests for patients with LCMM.

Establishment of a reference range for free light chains in elderly population

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Background. The quantitative assay of free light chains (FLCs) is a recently introduced commercial test reported to be sensitive and specific for detecting FLC diseases such as primary systemic amyloidosis (AL), light chain deposition disease (LCDD), nonsecretory multiple myeloma (NSMM), and light chain multiple myeloma. Aims. We evaluated the reference range of this test in the elderly population. Methods. We measured the concentration of κ and λ FLCs in the plasma samples of 884 elderly subjects aged over 65 years. Among the 884 subjects, those who had abnormal features (monoclonal peak in immunofixation, hypo or hyper-gammaglobulinemia, acute phase pattern C-reactive protein (CRP) of >1.0 mg/dL, and renal dysfunction Modification of Diet in Renal Disease (MDRD) glomerular filtration rate (GFR) of <60 mL/min/1.73 m2) were excluded. Levels of FLC were determined using the FLC assay (Freelite; The Binding Site, Birmingham, United Kingdom) performed on a Siemens Dade Behring BN II Nephelometer (Deerfield, IL). The assay consists of 2 separate measurements: one to detect free κ (normal range, 3.3-19.4 mg/L) and the other to detect free λ (normal range, 5.7-26.3 mg/L) light chains. The assay reports the FLC k/λ ratio (normal range, 0.26–1.65). The total FLC assay result was defined as an abnormal FLC k/λ ratio. Results. Of the 409 subjects included in our study, 206 (50.4%) were male and 203 (49.6%) were female. Their median age was 75 years (range, 65-96). The 95% reference range for k FLC, λ FLC, and k/λ FLC ratio were 10.0-49.4 mg/L, 13.7-59.5 mg/L, and 0.34–1.32, respectively.

Conclusions. Reference ranges were extended in elderly subjects compared with those established previously. This study suggests that the reference range of FLCs need to be established in the elderly population.
BNP and NT-proBNP markers in patients with MM and accompanying chronic heart failure (CHF). Material and methods: 45 patients are included in the analysis (m-15, f-30) with relapses or refractory MM for which were satisfied following conditions: (1) ECOG ≤ 2; (2) Anemia with Hb < 8.0 g/dL; (3) proved CHF; (4) basic therapy for CHF (inhibitor APF ± diuretic) was spent not less than within last 2 weeks and (5) the chemotherapy concerning MM was assumed. The patients with heart failure with New York Heart Association (NYHA) IV and/or the constant form of atrial fibrillation and/or heart diseases and/or a heavy arterial pathology didn’t include in this study. CHF was diagnosed on basis of standard criteria and the present of the left ventricular enlargement or systolic functional impairment by echocardiography, according to the European Society of Cardiology guidelines. The levels of NT-proBNP and a BNP-fragment in blood serum have been defined at the moment of enrollment in study by ELISA. The mathematical definition of threshold values of concentration of markers was spent by means of construction of ROC-curves and Kaplan-Mayer analysis. Results: The median of age of patients at the moment of enroll in study has made 66 (42-83 range) years. 3 (7%) patients had IA stage on Salmon-Dune and 22 (49%) - IIA and 20 (44%) - IIIB. 17 (38%) patients had no clinical signs of CHF. 16 (35%) patients have CHF with NYHA I and 9 (20%) - II and 3 (7%) - III. 28 (62%) patients have received salvage regimens of chemotherapy with bortezomb and 15 (33%) - with alkyating agents and 2 (5%) - high doses of dexamethasone. The objective response was documented for 26 (58%) patients including complete response (CR) or very good partial response (VGPR) - 7 (16%). 53 (73%) patients have alive at a median of follow up 11 months. The analysis of NT-proBNP levels has revealed correlation between the degree heart failure and a disease outcome (p < 0.05). Threshold concerning a failure of disease value of concentration NT-proBNP ≥ 300 ng/ml. The sensitivity of 83%, specificity - 62% has identified at concentration 0.93 ng/ml. For a BNP-fragment of authentic distinctions in the conditions of the limited sample has not received. Conclusions. The elevated serum NT-proBNP levels > 0.93 ng/ml is identified as the poor prognostic factor for patients this MM and accompanying CHF.

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LENALIDOMIDE PLUS DONOR LYMPHOCYTES INFUSION (DLI) AFTER ALLOGENEIC STEM-CELL TRANSPLANTATION (ALLO-SCT) WITH REDUCED-INTENSITY CONDITIONING IN PATIENTS WITH HIGH RISK MULTIPLE MYELOMA

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Relapse remains the main problem after allogeneic stem cell transplantation (Allo-SCT) in high risk Multiple Myeloma (MM) patients. The aim of our prospective study is to evaluate the anti-myeloma effect of lenalidomide followed of donor lymphocyte infusion (DLI) as adoptive immunotherapy after transplantation. Patients and methods. Twelve patients with refractory and high risk myeloma were analysed. Median age at transplantation was 56 years (46-64); 6 patients (50%) received lenalidomide before Allo-SCT. All patients received a RIC including Flu-darabine 30 mg/m² 5 days, ATG 2,5 mg/kg for 2 days and Busilvex 3.2 mg/kg/day (3 days in 6 patients and 2 days in 6 patients). All but one one received peripheral blood stem cells (PBSC). Donor was HLA id in 6 patients and matched unrelated in 6 patients. Patients were treated by lenalidomide if myeloma was progressive or residual disease was observed starting from day +100 and if no GVHD signs were evident. Dose was between 10 and 25 mg/day. DLI were administered after at least 2 cycles of lenalidomide. Results. The median time between Allo-SCT and lenalidomide was 10 months (3-58). The median initial dose of lenalidomide was 15 mg (10-25). The patients received a median of 6 cycles (1-10). 9 patients (60%) received an escalating dose of DLI; 1x10⁸/kg of CD3+ cells for the first DLI and 1 x 10⁸/Kg of CD3+cells for the second DLI. One patient with GVHD and two patients with progressive disease after lenalidomide did not receive DLI. The toxicity related to lenalidomide was haematological grade II in 4 patients (33%) and grade I in 3 patients (25%); 7 patients (50%) had moderate asthenia. one patient developed a reversible renal insufficiency after 10 cycles of lenalidomide, none of our patients developed thrombo-embolism under treatment. At the last follow up, 9 patients are alive and all of them are under ongoing treatment. Four patients achieved complete remission (CR) and live patients partial remission at last evaluation. The median of 2 years probability of the progression-free survival (PFS) was 75% and 50% and overall survival (OS) was 83% and 69% respectively. The median OS was not reached and the median PFS was 23 months. Conclusions: These data show that lenalidomide has an acceptable toxicity profile is well tolerated after Allo-SCT. Combination with DLI should be further evaluated in a larger cohort of patients.

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THALIDOMIDE-INDUCED SENSORY NEUROPATHY IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Chemotherapy-induced sensory neuropathies differ in clinical picture. There is predominance of paresthesiae in some of them while in others pain or deep sensation failure dominates. Aim. Clinical and electrophysiological assessment of peripheral sensory nerves in patients with multiple myeloma (m.m.) treated with thalidomide. Special attention was directed to function of subtypes of sensory fibres which convey different modalities of sensation. Material and methods. Twenty seven m.m. patients and 30 controls were examined. Neurological examination together with allocation to different groups acc. to sNCI-CTC scale were performed. Standard sensory conduction velocity was measured in ulnar and sural nerves. Quantitative Sensory Testing (QST) was used to determine thermal detection thresholds. Results. All patients informed about subjective positive sensory symptoms and sensory deficit of symmetrical, distal pattern was found. Electroneurography revealed axonal and demyelinating abnormalities with dominance of axonal injury. Warm and heat-pain detection thresholds were elevated, while threshold for skin cooling was decreased both in palm and foot in m.m. patients in comparison with controls. There were no differences in the thresholds for cold-pain detection between examined groups. Conclusions. Thalidomide-induced sensory neuropathy can appear shortly after the introduction of treatment. Patients with longer duration of treatment or with higher cumulative dose present higher degree of neuropathy acc. to the sNCI-CTC scale. Sensory deficit in thalidomide’ neuropathy is associated with dysfunction in A delta and C caliber primary afferent fibres.

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CORRELATION OF SERUM LIGHT CHAIN LEVELS AND IISH LIGHT CHAINS STAINING IN TREPHEINE BIOPSIES IN MULTIPLE MYELOMA PATIENTS: SOUTH AUSTRALIAN EXPERIENCE

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Background. The detection and quantification of monoclonal plasma cells in bone marrow biopsy is essential in the diagnosis of plasma cell neoplasms and in assessment of treatment response. Controversies exist relating to a standard for conventional immunohistochemistry staining (IHC) in the detection of Ig light chains, as interpretation is operator dependent and limited by heavy background staining. In situ hybridization (ISH) is an established technique in the detection of Ig light chain
mRNA, utilizing specific probes to demonstrate monoclonality in myeloma and B cell lymphomas.

**Aims.** This single centre retrospective review assesses the efficacy of this technique (ISH) by correlating with serum light chain levels. **Methods.** A random cohort of 20% of bone marrow biopsies performed between November 2009 and January 2011 for multiple myeloma was included in this review. The kappa and lambda positive cells in the marrow were quantified as a ratio of the percentage of kappa/lambda positive cells to the percentage of CD138 positive cells. **Results and conclusions.** The mean age of our patients was 65 years (range 38-85yrs) and 48% were female. The majority of patients had IgG kappa subtype (50%). A statistically significant correlation was demonstrated between serum lambda level and lambda positive cells in the trephine biopsy (R=0.9069, p<0.0001). There was no significant correlation between serum paraprotein level and kappa or lambda positive cells in trephine biopsy. Furthermore, six patients (15% - total 38 patients) were noted to have significant disease by CD138 and kappa/lambda staining despite having low plasma cell count (less than 10% by smear morphology and immunophenotyping). In conclusion, our study highlighted the greater sensitivity of ISH staining in comparison to conventional morphological criteria, with statistically significant correlation with serum free light chain levels and is therefore a useful tool in the diagnosis and follow up of myeloma patients. Further studies are needed to better clarify its role.
**1315**  
**IMMUNOGLOBULIN OLIGOClonAL BANDS AND ISotype SwITching AFTER Bone MARrow TRANSPLANTATION IN PATIENTS WITH Multiple Myeloma**  
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**Background:** Appearance of new monoclonal or oligoclonal immunoglobulin bands was reported to be common in myeloma patients after receiving bone marrow transplantation (BMT). Aims: We aimed to study this phenomenon in a cohort of Chinese patients who had received autologous or allogeneic BMT as part of the treatment of myeloma. Methods: The clinical records and laboratory results of 41 patients over a 6-year period (2005-2010) were reviewed retrospectively. Results: The distribution of the original monoclonal immunoglobulin bands was IgG (n=13), IgM (n=11), IgA (n=3), IgG/K (n=3), IgG/L (n=4), free light chain lambda (n=3), and free light chain kappa (n=2). Conclusions: This study demonstrates new monoclonal or oligoclonal immunoglobulin bands frequently develop after patients with myeloma receive BMT. Although not significant statistically the long term survival of patients who have new immunoglobulin bands appears more favourable than those who do not have new immunoglobulin bands. A larger cohort is needed to confirm these findings.

**1316**  
**PREVIOUSLY NOT DESCRIBED ASSOCIATION OF SKIN CRYOglobulinemia TO A SYSTEMIC CAPILLARY LEAK SYNDROME (SCS) WITH Multiple Myeloma**  
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**Background:** Appearance of new monoclonal or oligoclonal immunoglobulin bands was reported to be common in myeloma patients after receiving bone marrow transplantation (BMT). Aims: We aimed to study this phenomenon in a cohort of Chinese patients who had received autologous or allogeneic BMT as part of the treatment of myeloma. Methods: The clinical records and laboratory results of 41 patients over a 6-year period (2005-2010) were reviewed retrospectively. Results: The distribution of the original monoclonal immunoglobulin bands was IgG (n=13), IgM (n=11), IgA (n=3), IgG/K (n=3), IgG/L (n=4), free light chain lambda (n=3), and free light chain kappa (n=2). Conclusions: This study demonstrates new monoclonal or oligoclonal immunoglobulin bands frequently develop after patients with myeloma receive BMT. Although not significant statistically the long term survival of patients who have new immunoglobulin bands appears more favourable than those who do not have new immunoglobulin bands. A larger cohort is needed to confirm these findings.

**1317**  
**HOW LARGE A DIFFERENCE BETWEEN SERIAL MEASUREMENTS OF FREE LIGHT CHAINS IS NEEDED TO INDICATE A RESPONSE OR PROGRESSION IN PLASMA CELL DISORDERS?**  
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**Aims:** The distribution of the original monoclonal immunoglobulin bands was IgG (n=13), IgM (n=11), IgA (n=3), IgG/K (n=3), IgG/L (n=4), free light chain lambda (n=3), and free light chain kappa (n=2). Conclusions: This study demonstrates new monoclonal or oligoclonal immunoglobulin bands frequently develop after patients with myeloma receive BMT. Although not significant statistically the long term survival of patients who have new immunoglobulin bands appears more favourable than those who do not have new immunoglobulin bands. A larger cohort is needed to confirm these findings.

**Results:** The distribution of the original monoclonal immunoglobulin bands was IgG (n=13), IgM (n=11), IgA (n=3), IgG/K (n=3), IgG/L (n=4), free light chain lambda (n=3), and free light chain kappa (n=2). The intra-individual variation, CVb for κ and λ is 8.3% for κ and 24.9% for λ. We found a minimal and identical variation, CVb for κ and λ is 8.3% for κ and 24.9% for λ. The critical difference between two serial results to be significantly different (p < 0.05). Methods: FLC: were measured in serum of 7 healthy persons (mean age 41 (21-60) years, 2 males and 5 females), 6 patients with multiple myeloma (mean age 63.2 (44-73) years, 3 males and 3 females) and 5 patients with monoclonal gammapathy of unknown significance (mean age 66.2 (62-70) years, 1 male and 4 females). From each patient we collected 8 serum samples, 5 of these taken every morning (between 8 and 9 am) for 5 days (day-to-day variation). The serum samples were collected in PET tubes with gel. Samples were centrifuged, pipetted and stored at -20°C. All 8 samples from each patient were analysed in the same run, on the same instrument and by the same experienced technician. FLC were also measured in 17 serum samples with κ values from 15-11,000 mg/L and 15 serum samples with λ values from 14-516 mg/L to determine the imprecision. The critical difference is then calculated using the following formula: 2.77 (CVa2 + CVw2)½. Results: The intra-individual variation, CVw for κ and λ were found to be 6.8% and 7.4%. The inter-individual variation, CVb for κ and λ is 21.6% and 29.8%. The imprecision of the FLC assay, the coefficient of variation for the analysis, CVa, is 8.3% for κ and 5.1% for λ. The critical difference between two serial results to be significantly different is then calculated to be 29.7% for κ and 24.9% for λ. Summary/conclusions: We found a minimal and identical intra-individual biological variation for both healthy individuals and patients with monoclonal plasma cell disorders. The current FLC assay cannot meet the desirable laboratory performance standards, or analytical goals, for the imprecision of analytical methods derived from data on biological variation. The FLC analysis is showing great individuality, and the best reference for patients is their own former results. We calculated the critical difference between two serial results to be 29.7% for κ and 24.9% for λ, respectively, to be assessable as evidence of progression or response in plasma cell disorders.
**1318**

**TRANSFORMATION OF MGUS INTO LIGHT CHAIN DEPOSITION DISEASE WITH A FAVORABLE RESPONSE TO BORTEZOMIB TREATMENT**

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**Background.** Recent literature has described only rarely the transformation of MGUS into light chain deposition disease with successful treatment using bortezomib. **Methods and results.** Case description: A 24-year-old male has been followed for 10 years for MGUS IgG lambda. During the examination for weight loss (15 kg) and dyspnea we found considerable anemia (Hb 86 g/l), increased levels of creatinine (148 µmol/l), serum M-protein 11.6 g/l, increase of λ serum free light chains (FLC) to 542.2 mg/l (κ: λ index 0.045), suppression of normal immunoglobulin IgM (0.22 g/l), NT pro BNP 2818, slight focal increase of monoclonal plasma cells with expression of FLC -λandmbda, microscopic hematuria (294.0), proteinuria 2.1 g/24h (B-J-). Ultrasonography revealed kidney enlargement with hyperechogenity of the parenchyme, histology of the kidneys demonstrated linear FLC lambda deposits in the basal membrane of the tubules forming the diagnosis of LCDD type lambda. Spiral HR-CT and FDG-PET/CT displayed GGO of lower lung lobes and multiple lung parenchymal consolidations with left-sided pleural effusion. After 4 cycles of bortezomib and dexamethasone we could observe substantial improvement of overall condition, disappearance of dyspnea with pulmonary finding regression, normalization of blood count (Hb 132), decrease of M-protein (2.6g/L), FLC λ (25.8, κ:λ index 0.445), proteinuria (0.31 g/24h), creatinine (114 µmol/l), decrease of beta2-microglobulin (2.8-1.6 mg/l). **Conclusions.** We conclude that MGUS patients need a permanent follow-up that might enable to record the transformation into another monoclonal gammopathy, in this case a LCDD with successful bortezomib and dexamethasone treatment as an induction for autologous stem cell transplantation.

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**1319**

**PARTIAL ‘LIGHT-CHAIN ESCAPE’ PHENOMENON & CYTOGENETIC CHANGES - TWO CASE STUDIES**

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**Background.** The light-chain escape (LCE) phenomenon refers to the condition when, in the relapse/progression stage of multiple myeloma (MM), the secretion of monoclonal light chain becomes dominant to the condition when, in the relapse/progression stage of multiple myeloma

**Results.** Case 1: A 58-year-old patient with MM IgA-k IIIA, ISS II (MIG 50 g/l, κ 129 mg/l), (46,XY[23]; FISH: 3 copies of IgH, 1, 7; 5 copies of 1q21; polyom aty 9,17), who achieved very good partial remission (MIG 1,47, kappa 20) after induction treatment with CTD and the subsequent autologous transplantation of stem cells (HD-T/ASCT). The following early progression was characterized by partial LCE (MIG 4,8, kappa 1765) and changed cytogenetics (46XY[17];76-88[FISH: 2 copies of IgH, t(8;14); 3 copies of 1; 6-7 copies of 1q21; polyom aty 7, 9, 11, 17). The progression was associated with extramedullar proliferation and resistance to chemotherapy involving the administration of best carbopep and lenalidomide. Case 2: A 65-year-old patient with MM IgA-k, IIIA, ISS II (MIG 42, lambda 45), (46XX[9]; FISH: del RB1, t(4;14) achieved stringent complete remission (sCR) after induction treatment with CTD and the subsequent HD-T/ASCT. After 15 months, focal relapse with extramedullar proliferation was proven in the pelvis area, while sCR was achieved after local radiotherapy and chemotherapy with VCD again. After 4 months, systemic relapse of the disease was proven in the form of partial LCE (MIG 5.5, lambda 834) associated with karyotype evolution (48-45,XX,-2,-4,15,1-3mar[p9]; FISH: del RB1, t(4;14), del TP53. The relapse was connected with resistance to further chemotherapy, including the administration of lenalidomide.

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**1320**

**THE IMPACT OF VELCADE TREATMENT ON SURVIVAL OF RELAPSED MYELOMA PATIENTS – A SINGLE INSTITUTION EXPERIENCE**

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**Background.** According to SUMMIT trial, Velcade salvage for repeated relapsed myeloma patients showed the overall response to have 28%. A randomized phase III multicenter, APEX trial, compared velcade to high dose dexamethasone in 669 patients with relapsed/refractory myeloma patients revealed superior median overall survival (29.8 vs 23.7 months) for velcade. Methods: Between August 2005 and October 2010, nineteen relapsed myeloma patients underwent salvage velcade treatment at our institute and we compared the earlier 40 myeloma patients not received velcade. The endpoint is the overall survival in these patients with or without the use of velcade. Results. The median ages of velcade group and no-velcade group are 60.2 vs 61.7 (range 41–79 vs 39–80), respectively. The stage III disease percentages are 63.2% vs 55.0%. The median prior lines of treatment before velcade are 1.84. The median overall survivals of velcade group and no-velcade group are 60 vs 25 months (5-year survivals are 52.0% vs 15.5%). Conclusions -In our historical comparison between velcade and no-velcade groups of patients, velcade surely could prolong the overall survival of relapsed myeloma patients.

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**1321**

**MR-PROADM AS MARKER OF VASCULAR DAMAGE REVERSIBILITY IN AL AMYLOIDOSIS PATIENTS**

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**Background.** Light-chain AL amyloidosis is the most common form of systemic amyloidosis and is associated with an underlying plasma cell dyscrasia. Circulation dysfunction is very frequent in AL amyloidosis patients. Serum midregional fragment of pro-adrenomedullin peptide (MR-proADM) and free light chain (s-FLC) κ and λ levels were investigated in order to evaluate circulation impairment in systemic AL amyloidosis patients. **Summary.** The above mentioned observation confirms that the presence of LCE and the unfavorable karyotype evolution in the disease progression/relapse tend to be related to the subsequent adverse course of the disease and resistance to therapy. Determining the serum levels of free light chains and repeated cytogenetic examinations allow to recognize early relapse/progression, particularly in case of LCE, and to identify patients with adverse prognostic requiring an early intensive chemotherapy.

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lloidosis. MR-proADM has been described as an useful marker for heart, respiratory and circulating dysfunction assessment and its role as a prognostic factor we evaluated in patients with systemic diseases. Aims and Methods. MR-proADM and s-FLC levels were detected in 7 patients (median age 63.1 yrs) with systemic AL α amyloidosis at exordium observed to our Unit. Ten age-matched healthy control individuals were selected. According to age and disease risk stratification six patients were treated with upfront oral MelDex association (Melphalan 9 mg/sm, Dexamethasone 20 mg day 1,4,8,11 q21). One subject started first line therapy with BorDex association (Bortezomib 1.3 mg/sm, Dexamethasone 20 mg day 1,4,8,11 q21). Three samples of peripheral blood were performed (treatment day 1, day 8 and at conclusion of the first cycle of therapy). The blood was separated into plasma at the time of blood draw and frozen to -80°C. In the evaluation of results Mann-Whitney U test, paired t test, Kruskal-Wallis one-way analysis of variance (Dunn’s Method versus Control Group), Spearman rank correlation and Linear Regression were performed. P values ≤ 0.05 were considered statistically significant. Results: s-FLC λ values were significantly decreased during treatment (p=0.05). s-FLC κ/λ ratio and MR-proADM level were both increased at the end of first course of therapy (p=0.002 and p<0.05 respectively). On day 1 a positive correlation between s-FLC λ and MR-proADM level was observed (r=0.82, p=0.03). Conclusions. The reduction of s-FLC levels observed in patients with systemic AL amyloidosis is indicative of hematological response to treatment. On the other hand, the progressive increase of MR-proADM serum level could represent a warning for possible vascular damage even if haematological response has been achieved. Therefore MR-proADM could be considered an additional useful biomarker in the evaluation of systemic AL amyloidosis cases, more than one vertebra was treated in the same procedure. Pain response was evaluated by a qualitative scale at 24 hours, 1 and 6 months after PV. Results. Nineteen PV were performed in 15 patients (12 females and 3 males). Thirty-eight vertebrae were treated (maximum 4 vertebra in the same procedure), being the most frequent localization L3. Median age was 74.8 years (range, 39 to 88 years). The evaluation of pain at 24 hours, 1 and 6 months after PV, showed improvement in 79%, 47% and 37% of cases respectively. Notably, most patients reported pain in other skeletal localization caused by disease progression, but unrelated to treated vertebra. The incidence of cement leakage was 47%. Four out of 15 patients developed severe complications: 1 psoas hematoma without hemoglobin decrease in the first 24 hours after PV with good outcome, 1 death by respiratory failure of unknown etiology 11 days after PV and 2 pulmonary embolism (the first one died in the first 24 hours after the third PV because of a cement pulmonary embolism; the second one was hospitalized one week after PV and treated successfully with heparin). Conclusions. PV is an easy technique for SML not responsive to medical treatment that results in immediate pain relief in 79% of patients. Severe clinical complications secondary to cement leakage can be observed in 26% (4 out of 15) of patients, with some being life-threatening. These results suggest that PV can be useful in acute SML treatment to improve pain related but further studies with more patients and more follow-up should be undertaken to confirm the efficacy and the incidence of adverse effects.

1323 INTERNATIONAL STAGING SYSTEM PREDICTS PROGNOSIS OF CHINESE PATIENTS WITH MULTIPLE MYELOMA ACROSS DIFFERENT CALENDAR PERIODS WITH APPLICATION OF NOVEL AGENTS

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Background and Aims. The International Staging System (ISS) has been proposed since 2005 but the applicability of Chinese patients with multiple myeloma (MM) is not known, especially for those who had received treatments with novel agents. Methods. MM Patients who were newly diagnosed in Taipei Veterans General Hospital were enrolled between 1996 and 2007. Data of clinical features, laboratory tests and outcome at last follow up were collected. Results. Total 389 MM patients were enrolled, with median age of 71 years. Seventy-one percent of patients were male and more than 70% were older than 65 years. At diagnosis, patients had disease at Durie-Salmon (DS) stage III and ISS stage III were 72.7% and 56.2%, respectively; and those with serum creatinine Cr ≥ 2.0 mg/dl at diagnosis was noted in 34%. Comparing with those diagnosed in the first calendar period 1996-2001, patients of the second calendar period 2002-2007 were older and more had received novel agents, especially for thalidomide.

1322 PERCUTANEOUS VERTEBROPLASTY IN PATIENTS WITH SPINAL MYELOMA LESIONS

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Background. About 80-90% of patients with multiple myeloma will develop skeletal-related complications, including diffuse osteopenia, focal lytic lesions, pathological fractures and bone pain. The therapeutic intervention for spinal myeloma lesions (SML) is based on analgesic medications, bisphosphonates, radiation therapy, and in some cases, percutaneous vertebroplasty (PV) and balloon kyphoplasty. PV consists in the injection of polymethylmethacrylate into the damaged vertebral body via a percutaneous approach under image guidance. While in percutaneous vertebroplasty osteoclasts are killed, 2 and randomized trials have shown no beneficial effect of PV compared with placebo or a simulated procedure without cement, no specific data in controlled trials are available for myeloma patients. Aims. To evaluate the efficacy and safety of PV in patients with SML. Methods. Patients with SML not responsive to medical treatment were eligible. A computed tomography and/or magnetic resonance were performed, previously to the procedure. The presence of pathological fractures was evaluated by a rheumatologist and/or radiologist specialist and performed by an interventionist radiologist with local anesthesia and light sedation. In some cases, more than one vertebra was treated in the same procedure. Pain response was evaluated by a qualitative scale at 24 hours, 1 and 6 months after PV. Results. Nineteen PV were performed in 15 patients (12 females and 3 males). Thirty-eight vertebrae were treated (maximum 4 vertebrae in the same procedure), being the most frequent localization L3. Median age was 74.8 years (range, 39 to 88 years). The evaluation of pain at 24 hours, 1 and 6 months after PV, showed improvement in 79%, 47% and 37% of cases respectively. Notably, most patients reported pain in other skeletal localization caused by disease progression, but unrelated to treated vertebra. The incidence of cement leakage was 47%. Four out of 15 patients developed severe complications: 1 psoas hematoma without hemoglobin decrease in the first 24 hours after PV with good outcome, 1 death by respiratory failure of unknown etiology 11 days after PV and 2 pulmonary embolism (the first one died in the first 24 hours after the third PV because of a cement pulmonary embolism; the second one was hospitalized one week after PV and treated successfully with heparin). Conclusions. PV is an easy technique for SML not responsive to medical treatment that results in immediate pain relief in 79% of patients. Severe clinical complications secondary to cement leakage can be observed in 26% (4 out of 15) of patients, with some being life-threatening. These results suggest that PV can be useful in acute SML treatment to improve pain related but further studies with more patients and more follow-up should be undertaken to confirm the efficacy and the incidence of adverse effects.

1323 INTERNATIONAL STAGING SYSTEM PREDICTS PROGNOSIS OF CHINESE PATIENTS WITH MULTIPLE MYELOMA ACROSS DIFFERENT CALENDAR PERIODS WITH APPLICATION OF NOVEL AGENTS

LT Hsiao, SH Yang, HW Teng, YC Hong, CY Liu, YB Yu, JP Gau, JH Liu, PM Chen, TJ Chio, CT Zeng
Taipei Veterans General Hospital, Taipei, Taiwan

Background and Aims. The International Staging System (ISS) has been proposed since 2005 but the applicability of Chinese patients with multiple myeloma (MM) is not known, especially for those who had received treatments with novel agents. Methods. MM Patients who were newly diagnosed in Taipei Veterans General Hospital were enrolled between 1996 and 2007. Data of clinical features, laboratory tests and outcome at last follow up were collected. Results. Total 389 MM patients were enrolled, with median age of 71 years. Seventy-one percent of patients were male and more than 70% were older than 65 years. At diagnosis, patients had disease at Durie-Salmon (DS) stage III and ISS stage III were 72.7% and 56.2%, respectively; and those with serum creatinine Cr ≥ 2.0 mg/dl at diagnosis was noted in 34%. Comparing with those diagnosed in the first calendar period 1996-2001, patients of the second calendar period 2002-2007 were older and more had received novel agents, especially for thalidomide.

Figure 1. Survival curve of 351 MM patients by ISS.

The median overall survival was 20.5 months, with a significant increase in the second calendar period (15.3 and 28.2 months, respectively; P=0.002). In the Cox proportion model using those factors at diagnosis (except for DS and ISS themselves, and calendar period, those factors included elevated serum β2 microglobulin at diagnosis (>3.5), old age (>65 years), and impaired renal function were independently associated with a poor survival. For the whole period, the ISS is better than DS in predicting the prognosis. The prognosis of MM patients in stage I and II of the second calendar period is significantly better than those of first period; however, the difference is not significant for stage III.
Conclusions. The findings of our study is the first to show the applicability of ISS in Chinese patients with MM, especially for those who have received thalidomide.

**1324**

**EFFECTIVENESS AND SAFETY OF LENALIDOMIDE IN PATIENTS DIAGNOSED OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA WITH EXTRAMEDULLARY DISEASE**

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**Background.** Secondary extramedullary plasmacytoma (EP) is an aggressive form of plasma cell disorders characterized by tumour masses of malignant plasma cells outside of the bone marrow in patients diagnosed of multiple myeloma (MM). Different therapeutic approaches have been reported with poor outcomes. Our study reports the role of lenalidomide for EP in daily clinical practice. We analyzed the clinical data of 18 Spanish patients (pts) with refractory or relapsed multiple myeloma with extramedullary plasmacytoma EP treated with lenalidomide outside of clinical trials in 12 GEM/PETHEMA centers between October 2007 and July 2010. The treatment consisted on lenalidomide ranging from 10 to 25 mg, given on days 1-21 of a 28-days cycles, combined with dexamethasone. The response rate was evaluated according to the international criteria and the response of EP by measuring size changes by physical examination and/or radiological imaging. The median observation time was 12 months (3 - 24). Safety data were evaluated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events v3.

**Results.** Eighteen unselected patients (eight females, median age 68 years) were analyzed. A median of 3 previous lines of therapy (1 - 6) were given, including autologous stem cell transplantation (4/18), and novel agents such as bortezomib (18/18) and thalidomide (3/18). Median number of lenalidomide cycles administered was 7 (3 - 21) with a maximum response after a median of 3 cycles (2 - 10). The overall response rate (ORR) ORR of MM was 61.1%, (complete response (CR) 16.6%, very good partial response (VGPR) 22.2%), EP disappeared in 8/18, EP size decreased in 3/18. The progression free survival (PFS) and overall survival (OS) PFS and OS were 9.8 and 14.6 months, respectively. To date, 9/11 responding patients relapsed, and 10/11 patients are alive. Lenalidomide toxicity was predominantly hematologic (5/18) and the incidence of venous thrombotic events was low (1/18). Conclusions. Our results suggest lenalidomide could be an effective and manageable drug for patients with advanced myeloma with EP. A randomised trial is needed to assess the role of lenalidomide compared with other treatment options for secondary EP.

**1325**

**SURVIVAL ACCORDING TO END-ORGAN DAMAGE PATTERNS IN SYMPTOMATIC MULTIPLE MYELOMA**

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*aims*. This retrospective analysis was performed to evaluate the differences of survival according to end-organ damage (CRAB) patterns in symptomatic multiple myeloma patients. Methods. Between September 1995 and December 2009, 400 consecutive patients of symptomatic multiple myeloma were treated in the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients. The Cox proportional hazards model was used to assess prognostic significance of end-organ damage (CRAB) patterns adjusted for age, sex, performance status, autologous stem cell transplantation (ASCT), international staging system (ISS) stage and the use of novel agents. Results. The median overall survival was 30.0 months (95% CI 24.4-35.6) with a median follow-up period of 23.6 months (range, 0.2 - 184.8). The median age was 61 years (range 20-85). Male patients consisted of 55% (222/400). Calcium level increase (C, serum calcium >11.5 mg/dL) was present in 10% (40/398), renal insufficiency (R, serum creatinine >2mg/dL) in 24.5% (98/390), anaemia (A, hemoglobin 2 g/dL below the lower limit of normal or hemoglobin <10 g/dL) in 66% (264/392), and bone lesions (B, lytic lesions or osteoporosis with compression fractures) in 2% (5/265). ASCT was performed in 37% (149/392) patients (18/149) and 56.5% received novel agents at some time during treatment. In univariate analysis, ROTI patterns of R (C-R+A-B-), A (C-R+A+B-), RA (C-R+A+B+), RB (C-R+A-B+), AB (C-R+A+B+) and RAB (C-R+A+B+) were associated with shorter overall survival (OS) (p<0.01). In multivariate analysis, only R and RA patterns were prognostic for OS with marginal statistical significance (p=0.073, 0.082 and 0.090, respectively). However, R and RA patterns were significantly associated with poor prognosis in those who did not undergo ASCT (Hazard ratio 1.507, p=0.038; HR 1.694, p=0.016, respectively, Table 1).

**Conclusions.** Patterns of end-organ damage may be prognostic in symptomatic multiple myeloma patients. Renal insufficiency in particular seemed to correlate with poor prognosis and it was more evident in those who did not undergo ASCT. In contrast to renal insufficiency, hypercalcemia or bone lesions were not related to OS.

**1326**

**EMERGENCE OF OLIGOCLONAL BANDING IN PATIENTS WITH MULTIPLE MYELOMA AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION**

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*Background.* The emergence of oligoclonal banding in immunofixation of patients with Multiple Myeloma (MM) after autologous stem cell transplantation (ASCT) is a well-recognized feature of humoral response. This oligoclonal pattern appears to reflect an indirect sign of immune reconstitution and a robust humoral response with an uncertain clinical meaning. Aims. To address the clinical meaning of the oligoclonal banding presence in MM patients after ASCT. Prevalence of oligoclonal humoral response (OHR), progression-free survival (PFS) and time to relapse (TTR) were considered as outcomes. Methods: MM patients who underwent ASCT in our Hospital, from 2000 to 2009, were retrospectively analyzed. PFS and TTR were assessed using the International Myeloma Working Group criteria. An oligoclonal humoral response was defined by a serum immunofixation (IFE) different either in heavy or light chain component from the original monoclonal protein resulting in multiple banding providing an typical oligoclonal serum IFE pattern. Serum analysis for immunoglobulins and other biochemical markers was performed. Results were expressed as median (range). Results: Sixty five patients (31 female, median age 56yr, median follow-up 12 months) were studied. The overall survival was 58.5% (95% CI, 51.9-65.1%) and 86 patients had relapsed. Thirty-five (53.8%) developed an oligoclonal humoral response after ASCT, 3 episodes of OHR was observed in 2 patients and 2 episodes of OHR in 7. Oligoclonal...
humoral response was observed for 7 (1-37) months and in some cases persisted beyond relapse (5 patients). This response occurred with a 4 months increase in patient’s survival. Interestingly, relapse occurred simultaneously with oligoclonal banding disappearance in 4 patients. Conclusion: Our results are consistent with the hypothesis that disappearance of oligoclonal pattern is a hallmark of an early relapse and may be used as a surrogate marker of response in patients with MM after ASCT.

**1327**

**AN AUDIT OF REVLIMID (LENALIDOMIDE) USAGE, MONITORING AND TOXICITY IN MYELOMA IN A SINGLE UK HEMATOLOGY CENTRE**

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Background. Myeloma has a median overall survival of 3-5 years from diagnosis. New drug therapies, such as Revlimid are now available with results from clinical trials (MM-009/MM-010) indicating a significant improvement in PFS and OS compared to conventional therapy. Following NICE guidance in June 2009, Revlimid has been used in relapsed myeloma patients after two or more lines of previous therapy. Regional East Midland (EM) Guidelines have also been written on the effectiveness and toxicity of Revlimid is limited outside of clinical trials and this study looks at usage at Nottingham University Hospitals (NUH) NHS trust, a large UK Haematology centre, which has treated the highest number of relapsed myeloma patients with Revlimid in the UK to date. Aims. To audit the use, response rates and toxicity of Revlimid in NUH myeloma service and EM guidelines. Methods. A retrospective analysis of all patients initiated on Revlimid prior to September 2010 at NUH were audited using criteria defined by NICE(TAI171) and EM guidelines. Case notes and online records were used to obtain information. Audit criteria included the indication for initiation of therapy, monitoring of response and continuation of therapy, frequency and management of grade III and IV haematological and non-haematological toxicities (as per NID CTIC), initial dosing and modification, management of disease progression and all cause mortality. Results. The records of 78 patients, 37 female and 41 male, were audited. No females were of child-bearing potential. Median age of patients starting Revlimid was 70.5 years (range 42-87). All patients received Revlimid within its licensed indication. 76/78(97%) received it within NICE criteria. Median number of previous therapies was 3 (range 1-10) with 44/78 (56.4%) patients having had previous ASCT. Median number of cycles of Revlimid received was 7 (range 1-20). 47/78 (60.3%) patients received at least 6 cycles of treatment with 19/78 (24.3%) still on treatment after 12 cycles. 31/78 (39.7%) stopped prior to 5 cycles; 11 due to progressive disease and 14 due to toxicity. Dose was modified in 39/78 (50%) patients; 16 reduced due to declining renal function and 19 reduced due to haematological toxicity. Maximal response was achieved at a median of 3 cycles (range 1-10) and was CR in 8/78(10.3%), VGPR in 6/78 (7.7%), PR in 36/78 (46.2%) and SD in 19 (24.4%). 52/78 (42.3%) suffered grade III or IV haematological toxicity and 3/78 (3.9%) suffered thrombocytopenia II/IV grade in 4 patients (26%). Thirteen patients (87%) needed G-CSF, 4 patients (26%) needed blood and platelet transfusion. Extra-haematological toxicity was: neuropathy I/II grade in 6 patients (40%), 2 case of FUO (fever of undetermined origin). One patient had deep venous thrombosis treated with therapeutic dose of subcutaneous low-molecular weight heparin. Conclusions. This preliminary experience suggests that LENALIDOMIDE in combination with Liposomal Doxorubicin, and low dose of dexamethasone (RDd) seems to be efficacy and safety in relapsed/refractory myeloma patients.

**1329**

**SEQUENTIAL THERAPY WITH VALD, BORTEZOMIB AND CYCLOPHOSPHAMIDE AND HIGH DOSE MELPHALAN AS INDUCTION THERAPY FOR MULTIPLE MYELOMA, FOLLOWED BY MAINTENANCE THERAPY WITH BORTEZOMIB AND INTERFERON**

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Background. High-dose Melphalan and autologous stem cell transplantation (ASCT) is the standard therapy for Multiple Myeloma (MM) patients younger than 65 years even allogeneic Haemopoietic Stem Cells (HSC) donor is available. In multivariate analysis the Complete Remission (CR) before high-dose therapy is a significant independent variable for Overall Survival (OS) and Time To Progression (TTP). The advantages of a maintenance therapy and association with new drugs in a multi-step therapy is still under investigation. Aims. We tested the efficacy and toxicity of sequential therapy with Vincristine, Pegylated Liposomal Doxorubicin and Dexamethasone (VALD) followed by Bortezomib and Cy clophosphamide to obtain a CR before high dose of Melphalan and ASCT. The role of association Bortezomib and INF-α and to investigate MALPFA (α-2-IFN) as maintenance therapy was also investigated. Methods From September 2006 to June 2010 we treated 19 consecutive pts (6 M, 13 F) with new diagnosed symptomatic MM. Median age was 53 years (34-64). Thirteen pts had measurable M-protein: 6 presented IgG type and 5 IgA type. Two pts had monoclonal and 4 micromolecular disease. International Staging System (ISS) score was 1 in 15 pts, 2 in 3 pts and 3 in 1 pt. All pts presented bone marrow plasmacytosis > 30% and >19 pts an extensive bone disease. Patients received every 21 days VALD regimen (V 2 mg, AL 40 mg/m2 at day 1 and Dexamethasone 40 mg day 1-4) for 3 cycles and 3 courses of Bortezomib (1.3 mg/m2) on days 1 and 8 every 21 days. VALD is followed by Cyto phosphate (CP) at the end of VALD and before and after ASCT for HCS harvesting. On average 45 days later pts underwent to ASCT after infusion of Melphalan 200 mg/m2 as conditioning regimen. The maintenance therapy with Bortezomib (1.3 mg/m2 every 21 days) was assosiated...
ciated to α-2-IFN (1.5 megaU/twice a week) for two years. α-2-IFN alone was administered at the same dosage until disease progression. Results All patients achieved Partial Remission (PR) after VALID scheme, 6/19 pts and 8/19 pts obtained a CR and a Very Good Partial Remission (VGPR) respectively after Bortezomib therapy with Overall Response Rate 75.7%. Three pts with a PR and 1 pt in early relapse after Bortezomib were excluded from the study. After Cyclophosphamide four patients received Bortezomib and HCS and they harvested the CD34+ preset target (10x106/kg). Toxicity consisted in: 15 % WHO grade I-II neuropenia and 20% WHO grade I-II peripheral neuropathy. Two pts died for pneumonia, the first one after 2nd VALD cycle and the second one due to autologous transplantation (pts showed ISS score 2 and 3, respectively). All 13 pts submitted to ASCT obtained a CR and 11 of them obtained a continuous maintenance therapy. After 33 months (range 7-46) of median follow-up 7/11 pts (63.6%) are in continuous CR. Conclusion The sequential therapy with VALD, Bortezomib and Cyclophosphamide, followed by high dose of Melphalan and maintenance therapy with Bortezomib-α-2-IFN is effective and low toxic as up-front therapy in MM and could be experienced in a larger number of patients.

1330
SIMILAR TOXICITY AND LOWER EFFICACY OF ALTERNATING WITH RESPECT TO CONTINUOUS LOW-DOSE THALIDOMIDE FOR RELAPSED OR REFRACTARY MULTIPLE MYELOMA PATIENTS

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Background. Thalidomide is still a valid therapy for relapsed/refractory multiple myeloma (MM) patients. Prolonged exposure represents a risk factor for peripheral neuropathy (PN) occurrence. Aims. This study explores the feasibility of an alternating with respect to the standard continuous low-dose thalidomide scheme. Methods. Twenty-three MM pts relapsed refractory after at least one previous line of therapy were consecutively enrolled in a randomized multicenter two-arm trial. The study was performed according to the Declaration of Helsinki and approved by the ethics committee of each participating institution. Patients with baseline grade 2 NCI PN were not eligible. Pts received Thalidomide 100 mg on days 1-28 of each 28-day cycle in arm A (continuous treatment) and Thalidomide 100 mg on days 1-14 of each 28-day cycle in Arm B (alternating treatment). Oral Dexamethasone (20 mg on days 1, 8, 15, and 22 of each cycle) was added to both treatment arms for the first six cycles then stopped if registering at least a partial response (PR). Thalidomide was given in both arms until progression or significant toxicity occurred. Additional neurologic assessment was performed with clinical neuropathy scores and nerve conduction studies at baseline, every six months the first 2 years and then every six months until neurotoxicity resolution or end of follow-up for any cause. Results. Ten patients were randomized in arm A, thirteen in arm B. Patients in both arms had similar baseline characteristics. Twenty pts (86%) were in relapse after one previous chemotherapy. One patient had baseline grade 1 PN. The median follow up was 42 months (range 3-49 months). Twenty-two out of 23 pts were evaluable for response (there was one early-death for myocardial infarction). ORR was 77% (CR+VGPR 18%, PR 59%). Median time to best response: 5.7 months. OS from therapy starting was 44 months with 10 pts alive (43%). Sixteen pts progressed (73%), median FFS was 24.8 months. Arm A correlated to better responses and outcome. All patients in arm A were responsive (100%) with respect to 58% in arm B (p=0.03). In arm A there were 1 CR (10%), 2 VGPR (20%), 7 PR (70%), in arm B there were 1 CR (8%), 6 PR (50%), 5 NR (42%). Median FFS was 42 months in Arm A vs 7 months in B (p=0.02). Median OS was not yet reached in arm A compared to 24 months in Arm B. Seventeen pts (74%) developed PN: 3 pts PN was in arm A and 14 pts in ARM B (p=0.15) and time to PN occurrence (7.7 in arm A vs 5.6 months in arm B, p=0.5) were not statistically different in the two arms. Conclusions. Continuous low-dose thalidomide seems to give better results with a similar neurological toxicity to the alternating scheme.

1331
FIVE YEAR EXPERIENCE OF BORTEZOMIB FOR RELAPSED MYELOMA IN A REGIONAL CANCER NETWORK - THE ADDITION OF CYCLOPHOSPHAMIDE TO BORTEZOMIB IMPROVES RESPONSE RATE BUT NOT TIME TO NEXT TREATMENT

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Background. The novel agent bortezomib has become a standard of care for relapsed myeloma within the UK. This followed National Institute of Clinical Excellence (NICE) guidance in 2007. However it is not certain whether bortezomib’s effect is enhanced when given in combination with cyclophosphamide. Aims. We aimed to retrospectively determine the effect of bortezomib at relapse when given within a regional cancer network. At clinicians’ discretion, a cohort of patients also received cyclophosphamide in combination with bortezomib-steroid. In addition to determining response rate and time to next treatment, we also sought to establish the frequency of drug related side effects which limited treatment delivery. Methods. We audited the outcome of 118 myeloma patients identified chronologically who received treatment with bortezomib at first or subsequent relapse in the Peninsula Cancer Network, UK, between April 2006 and July 2010. Hospital records, chemotherapy prescription forms and pathology systems were retrospectively used to provide audit data. Results. Median age at diagnosis was 61.6 (range 41.2-88.4), paraprotein class IgG (51%), IgA (50%), IgD (1%), light chain disease (18%). The median time from diagnosis to first receiving chemotherapy was 0.4 months (range 0-110.4), 39% of patients (n=46) received a melphalan autograft after initial chemotherapy. The median time from diagnosis to receiving bortezomib was 31.3 months (range 1.5-143.2), 60% received bortezomib at first relapse, 17% at second relapse, and 15% at third or subsequent relapse. 65.3% (n=77) received bortezomib alone or in combination with prednisolone or dexamethasone. 54.7% (n=41) received cyclophosphamide in addition to bortezomib-steroid at clinician discretion. Both groups received a median of 4 bortezomib containing treatment cycles (range 1-8). The overall response rate (ORR=CR+PR) was 56.1% (n=24) in patients who received cyclophosphamide-bortezomib-steroid compared to 59% (n=29) in the non-cyclophosphamide cohort (p=0.03) - CR 12.2%, PR 45.9% versus CR 10.4% and PR 51.2% in the cyclophosphamide vs no cyclophosphamide group respectively. Fewer patients receiving bortezomib with cyclophosphamide (2.4% versus 11.7%) ceased treatment because of loss of initial treatment response, although this did not reach statistical significance (p=0.16). There was no statistically significant difference in the median time to next treatment: 11.1 months for the bortezomib-steroid cohort, compared to 9.9 months for those receiving additional cyclophosphamide (p=0.502, Mantel-Cox test). 33.9% of all patients stopped treatment prematurely due to side-effects, mainly peripheral neuropathy (18.6% of all patients), indicating the difficulty in tolerating the regime, even if a response is occurring. The rates of treatment limiting neuropathy were similar in those receiving cyclophosphamide (17.1%) compared to bortezomib alone (20.8%). Six patients (5%) died whilst receiving treatment. Summary/Conclusions. In this retrospective audit, although the addition of cyclophosphamide to bortezomib produced a higher ORR, it did not result in an increase in the time to next treatment. Arguably the strategy of choosing myeloma treatments by best ORR also needs to consider the duration of the response. This study also demonstrates that more than one-third of patients needed to stop bortezomib therapy prematurely due to side-effects, mainly neuropathy - highlighting the difficulties of bortezomib delivery in a real world clinical setting.

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COMBINATION OF BORTEZOMIB, MELPHALAN AND PREDNISONE IN RELAPSED/REFRACTORY MULTIPLE MYELOMA

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ATYPICAL CASE OF POEMS SYNDROME WITH A GOOD RESPONSE TO LENALIDOMIDE - DEXAMETHAZONE

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Poems syndrome is a rare disease characterized by peripheral neuropathy, skin lesions, organomegaly, endocrinopathy and monoclonal gammopathy. No effective treatment is available. We describe here a case of an atypical presentation of POEMS syndrome with response to lenalidomide. A 29 years old man was referred to our department due to anemia. Clinical examination revealed pale skin and maxillary lymph nodes in both maxillas, splenomegaly and ascites. Laboratory exams revealed an anemia, hypochromic microcytic, increase ESR, low iron levels and low TIBC levels with normal levels of ferritin and hypothyroidism. Lymph node biopsy revealed Castlemann's disease. The patient was treated with etyropoetin and methylprednisolone. Two months later peripheral neuropathy was noted and a decrease of left upper arm occurred. The patient was admitted to the university hospital for evaluation. Detailed evaluation revealed, monoclonal gammopathy, and skin rash, while splenomegaly persisted despite the treatment with steroids. The patient was initially treated with 6 cycles of rituximab without any improvement. The patient was subsequently treated with Lenalidomide (25 mg/2nd day) and Dexamethasone 40 mg every week. A significant response was occurred after 2 months of treatment. Correction of anemia with normalization of red cell parameters, disappearance of monoclonal gammopathy, resolve of skin rash and decrease of splenomegaly was occurred. The patient continue the treatment and after 6 months of treatment is in a very good clinical situation. Conclusions: Poems syndrome is a rare disease with no specific treatment. Increased levels of VEGF and neoangiogenesis has been proposed as the pathogenetic mechanism. Different kinds of treatments (steroids, melphalan, monoclonal antibodies (rituximab)) have been used without success. Lenalidomide plus dexamethazone may be an potential treatment acting against VEGF; as we can propose, according to the results observed to our patient.

1334 THE EFFICACY AND SAFETY OF BORTEZOMIB, MELPHALAN PLUS PREDNISONE IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS NO ELIGIBLE FOR TRANSPLANTATION

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Background. Bortezomib plus melphalan-prednisone (VMP) is an effective therapy as first-line treatment in patients with MM ineligible for stem cell transplantation. Aim: To assess the efficacy and safety of VMP regimen in patients with MM previously treated with a line of treatment. Patients and methods: 32 patients with relapsed/refractory MM received nine 6-week cycles of VMP: bortezomib (1.3mg/m2 days 1,4,8,11,22, 25, 29, 32, cycles 1-4; and days 1, 8, 22, 29, cycles 5-9) plus melphalan (9mg/m2) and prednisone (60 mg/m2) days 1 to 4, cycles 1-9. Response rates were measured using IMWG criteria and toxicities were assessed by CTCAE, v3.0. Results: Median age was 70 years (range 42-83) and included 15 male (47%). Disease characteristics at the time of diagnosis were: ISS I 39%, II 26%, III 55%, ECOG ≥ 3 in 17% and 21% of patients presented impaired renal function. Median number of prior lines therapies were 2 (range 1-6). Previous schedules received were: conventional poliquimiotherapy 45%, alkyl agents 45%, bortezomib 48%, IMIDs-containing regimens 35% and ASCT 12%. With a median follow-up of 6 months (1-35) response rates could be evaluated in 20 patients (63%) after a median of 3.5 cycles (1-9) received. Overall response rate was 70% (20% CR, 5% nCR, 15% VGPR and 30% PR). OS was 87.5%. Most frequent grade ≥3 AEs included: anemia 16%, neutropenia 16%, thrombocytopenia 12%, anemia 7%, thrombocytopenia 7% and 10% gastrointestinal symptoms. Peripheral neuropathy was reported in 45% of patients, 15% grade 3-4. Seven (11%) patients had VZV infections requiring dose adjustment of bortezomib, 24% of melphalan and 7% of prednisone. Treatment was discontinued in 22 patients (36%), due to toxicity in 14. Overall survival was 87%. Conclusions: VMP is an effective and well tolerated treatment option for patients with newly diagnosed MM who are not candidates for intensive therapy. Our results are similar to those described in previous studies and requiring a longer follow-up for confirmation.

1335 THE OPG/RANKL SYSTEM IN MYELOMA BONE DISEASE

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Aims. The aim of this study was to assess the implications of serum levels of OPG (osteoprotegerin) and sRANKL (soluble receptor activator of nuclear factor-κappa B ligand) in the process of bone remodeling in myeloma bone disease. Methods. The study was performed one group of patients with myeloma bone disease (n=9) compared with a control group (n=6, healthy patients). The serum levels of the bone markers were quantified by using ELISA (enzyme-linked immunosorbent assay) method. Results: In group with myeloma bone disease the serum level of OPG were 32.55±2.65pg/ml (p<0.001), those of sRANKL were 47.53±4.25pg/ml (p<0.001), and OPG/sRANKL ratio has the average of 1.198±0.134. Conclusions: The serum levels of OPG in myeloma bone disease decreased, secondary to the stimulation of osteoclastic apoptosis. These high levels of sRANKL certify the activation of osteoclasts with secondary increased bone resorption in myeloma bone disease. In this study we demonstrated a significant reduction of OPG/sRANKL ratio in group with myeloma bone disease, favoring osteolysis appearance, in comparison with the control group.

1336 BORTEZOMIB, ADRIAMYCIN AND DEXAMETHASONE IS SUPERIOR TO VAD-TYPE CHEMOTHERAPY: THE USE OF A NOVEL STATISTICAL METHODOLOGY IN THE PAD IRELAND TRIAL

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Background. The PAD-Ireland Trial compared the use of bortezomib, adriamycin and dexamethasone (PAD) with coordinated VAD chemotherapy (vincristine, doxorubicin and dexamethasone) in newly diagnosed patients with untreated multiple myeloma (MM). While a significant improvement in OS was observed with the PAD regimen, the reasons for this improved survival were not clear. One possible reason for the difference in OS between the two arms could be the inclusion of patients with high risk (H-RM) and standard risk (S-RM) disease on the chemotherapy arms. To investigate this issue we undertook a re-analysis of the PAD-Ireland data using a novel statistical methodology: the Padova-based survival analysis (PBSA) method for OS. Methods: OS was calculated from the date of diagnosis to the date of death or last follow-up. The PBSA method was applied to the PAD-Ireland data. Results: A significantly longer OS was observed in the PAD arm compared to the VAD arm (HR 0.68; 95% CI 0.50 - 0.94; p = 0.022). Subgroup analysis showed that the difference in OS between the two arms was of a similar magnitude in both S-RM and H-RM disease. Conclusions: PBSA analysis demonstrated that PAD was superior to VAD in the PAD-Ireland Trial and that the increased OS in the PAD arm was not due to the inclusion of a higher number of patients with H-RM disease.
Background. Bortezomib with adrijamycin and dexamethasone (PAD) is a highly effective induction combination with response rates of up to 95% (Oakervee et al, BJH 2005; 129:755-62). In this study patients who had received VAD/VAD-like regimen acted as their own controls and were given further treatment using the PAD regimen and compared using appropriate statistical methods. Aims. To test the efficacy of bortezomib, adrijamycin and dexamethasone combination therapy utilising a novel statistical analysis incorporating the patients own initial response to VAD as an internal control. Methods. This was a Phase 2 study with 3 cohorts of 23 patients. Cohort 1 was patients treated with VAD and auto transplanted, cohort 2 similar patients but not transplanted and cohort 3 patients refractory to VAD who proceeded directly to PAD without any intervening chemotherapy. The paraprotein level at the start of each type of treatment (VAD or PAD) was used as a measure of response. Results. Using EBMT criteria with addition of VGR, 7 patients in cohort 1 achieved CR after PAD compared to one patient achieving CR after VAD. Using the exact McNemar significant probability comparison of all responses gave p=0.0078 and using the Wilcoxon Signed Rank test p=0.002 in favour of the PAD therapy. Data on overall survival and toxicities will be presented. Conclusions. PAD was demonstrated to be significantly superior to VAD particularly in the refractory group. This type of study is an alternative to large phase 3 studies

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RETROSPECTIVE STUDY OF MONOCLONAL GAMMOPATHIES DETECTED IN THE CLINICAL LABORATORY IN A CANARIAN ISLAND: 9-YEAR SERIES


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Introduction. We studied the incidence, classification and isotype distribution of monoclonal gammopathies in our clinical population. Methods: Data was selected from routinely ordered serum protein electrophoresis (SPE), between 01/01 and 12/10, were analysed, looking for identification of pathological results. Age-specific prevalence rates for El Hierro island data were calculated by dividing the number of participants with MGUS in each age stratum by the number of participants in that stratum. The age-adjusted incidences were standardized with respect to the WHO World Standard Population Distribution, based on the world average population between 2000 and 2025. The clinical diagnosis was recorded from the patient case history. Results: We examined the results of 2449 SPE registered during this period. M-protein with either SPE or IFE as well as sFLC results were detected in a total of 29/609 >18 years-old patients; all of them had been diagnosed within this period, representing a 2,76% prevalence in our population. The mean age-adjusted incidence of monoclonal gammopathy was 7.56 per 100,000 inhabitants/year, ranging from 2,31/100,000 in 2001 to 9,25/100,000 in 2009. The median patient age at diagnosis was 68 years (range 27-91 years), with males accounting for 41% of all cases of monoclonal gammopathy. A 76% of the patients were clinically defined as presenting monoclonal gammopathy of undetermined significance, and 24% presented multiple myeloma. The most frequent M-protein isotype was IgG (668%), followed by [a (17%) and B] protein (10%). Conclusion: Many of our community hospital patients are over 50 years of age and present with non-specific symptoms potentially related to disorders associated with an abnormal EEF requiring laboratory evaluation and in which the clinical haematological analysis should play an important role in the diagnosis of MGUS.

1339
COEXISTENCE OF MULTIPLE MYELOMA (MM) AND MYELOPROLIFERATIVE OR AUTOIMMUNE DISORDERS; A SINGLE CENTER EXPERIENCE

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Background. The simultaneous occurrence of multiple myeloma (MM) and myeloproliferative (MPD), lymphoproliferative (LPD) or autoimmune disorders (AD) is extremely rare. Aims. To report the characteristics of patients presenting coexisting MM and MPD, LPD or AD and to discuss the possible biologic mechanisms involved. Methods: Medical files from 297 MM patients diagnosed and followed in our section from 1999 to present were reviewed. Results. 15 patients that presented another MPD, LPD or AD together with multiple myeloma, were identified. Among them, 9 were females and 6 were males. Their median age was 55 years (range 25-70). 5 of the studied females had presented the other disorder (one chronic myeloid leukaemia [CML], one chronic lymphocytic leukaemia [CLL], one Hodgkin lymphoma [HL], one follicular lymphoma [FL], one essential thrombocytosis [ET], one systemic lupus erythematosus [SLE]) prior to MM; their MM was asymptomatic, of IgG type and preceded by a documented IgG-MGUS in 5 of them; the 6th patient and light chain (kappa type) MM was preceded by a renal present of multiple myeloma. The remaining 3 females (all asymptomatic and of IgG type) presented the second disease (two idiopathic thrombocytopenic purpura [ITP], one Erythroleukemia) after MM; the patient with
Bone disease is present at diagnosis in almost all patients with MM and decreased bone mineral density is also frequently observed. Available clinical data indicates that bortezomib has a positive impact on bone health through a bone anabolic effect. We prospectively analyzed sequential bone densities from 11 patients with MM (2 smoldering and 9 relapsed cases), treated with single agent bortezomib without use of biphosphonates. Dexscan was obtained at baseline and after treatment median time of bortezomib exposure of 6 months ranging from 2 to 12 months. We compared T-score changes at lumbar spine and at femoral neck with micro-CT analysis of bone marrow biopsies obtained at the time points of the radiological studies. With a median age of 63 years, 9 males and 2 females were enrolled. At baseline mean Lumbar Spine and femoral neck T-scores were -1.08 and -1.9 respectively. After bortezomib exposure the mean Lumbar Spine T-score was -0.9, and the Femoral Neck T-score was -1.5 with mean positive changes in lumbar T-score of 0.3 and at femoral neck of 0.57. Five patient’s slides from routine diagnostic core biopsy samples were digitally scanned with the Aperio XT system (Vista CA USA). The entire hematopoietic area and bony trabeculae were identified using the gene pattern recognition algorithm and each compartment was quantified. The calculated trabecular area was then divided by total surface area to obtain trabecular volume (TV). The change in mean lumbar spine bone density (0.38), and the change in mean femoral neck bone density (1.1) were statistically significant (p=0.05, p=0.04 respectively). At the same time the change in femoral trabecular volume (25.5) was also found statistically significant (p=0.02 paired t-test). This pilot study has confirmed that patients treated with single agent bortezomib experience a significant increment in bone mineral density at lumbar spine and at femoral neck even after two months of therapy and for the first time we were able to report parallel statistical changes in TV by micro-CT analysis of routine diagnostic bone marrow biopsies.

### 1340

**THE RELEVANCE OF THE INTERNATIONAL STAGING SYSTEM IN MULTIPLE MYELOMA IN THE ERA OF NEW TREATMENT MODALITIES**

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**Background.** The International Staging System (ISS) was established and validated, providing consistent risk distinction of multiple myeloma (MM) patients (pts). The aim of study was to analyze the relevance of ISS score in the first relapse of MM patients treated with thalidomide or bortezomib-based regimens. Patients and methods. The study included 53 MM pts (27 male/26 female, mean age 56 yrs, range 38-81) in the first relapse of myeloma. IgG myeloma was diagnosed in 38pts, IgA in 7pts, IgD in 1pts, light chains in 6pts and non-secretory in 1pts. According to the clinical stage (CS, Durie-Salmon), patients were distributed as follows: II 14pts; III 39pts. Regarding ISS score, the grouping was performed in 6pts: ISS1 12pts; ISS2 14pts; ISS3 27pts. Renal impairment was present in 6pts. Thalidomide based regimens (Thal-Dex 13pts; CTD 7pts; TCED 5pts) were applied in the group of 25pts (mean no.6 cycles, range 2-8-cycles), while 26 pts were treated with bortezomib based therapy (Vel-Dex 26pts; CVD 1pts; MPV 1pts, mean no.6 cycles, range 2-8-cycles). Results: In the group of pts treated with thalidomide based regimens, positive treatment response (CR+VGPR+PR+MR, EBMT criteria) was achieved in 18/25pts. According to the ISS score, there was no significant difference (Fisher test, p=0.378) in the treatment response between high-risk pts (ISS3: 19/25 pts) and myeloma pts of low- and intermediate risk (ISS1:ISS2: 12/25pts). High-risk pts had significantly shorter duration of progression-free interval (mean: ISS3 26.8m vs. ISS1:ISS2 9.26m, Mann-Whitney test p=0.021). Median overall survival for this group of pts was 81m (range 14-100m). In terms of the overall survival, no difference (Log-rank test p=0.175) was found between pts with ISS3 (median 62m, range 18-120m) and pts with ISS1 and ISS2 (median 48m, range 14-114m). No significant differences were found in the analyzed regimens. In the second group of pts treated with bortezomib based therapy, treatment response (CR+VGPR+PR+MR, EBMT criteria) was achieved in 25/28pts. Still, there was no difference (Fisher test, p=0.5) in the treatment response between pts with ISS3 (13/28pts) and pts of low- and intermediate risk (ISS1:ISS2: 12/25pts). No difference was found in duration of the progression-free interval (mean: ISS3 25.8m vs. ISS1:ISS2 26.5m, Mann-Whitney test p=0.641). Median overall survival for pts treated with bortezomib based therapy was 72m (range 18-120m). Patients with ISS1 and ISS2 had significantly longer overall survival (Log-rank test, p=0.019) comparing to pts with ISS3 (ISS1:ISS2: median 64m, range 42-120m vs. ISS3: median 90m, range 18-96m). Conclusion: The ISS score, as a surrogate marker of MM activity, is of relevance for the relapsed myeloma patients in terms of “tailored-treatment”approach in the era of new treatment modalities. According to the ISS score, the notified influence of thalidomide- and bortezomib-based treatment on duration of progression-free interval and overall survival of relapsed myeloma patients indicates these agents as promising constituents of induction and maintenance therapy.

### 1341

**BONE DENSITY CORRELATES WITH MICRO-CT TRABECULAR VOLUME CHANGES IN ROUTINE DIAGNOSTIC BONE MARROW SAMPLES IN MULTIPLE MYELOMA (MM) PATIENTS AFTER BORTEZOMIB EXPOSURE**

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Bone disease is present at diagnosis in almost all patients with MM and decreased bone mineral density is also frequently observed. Available clinical data indicates that bortezomib has a positive impact on bone health through a bone anabolic effect. We prospectively analyzed sequential bone densities from 11 patients with MM (2 smoldering and 9 relapsed cases), treated with single agent bortezomib without use of biphosphonates. Dexscan was obtained at baseline and after treatment median time of bortezomib exposure of 6 months ranging from 2 to 12 months. We compared T-score changes at lumbar spine and at femoral neck with micro-CT analysis of bone marrow biopsies obtained at the time points of the radiological studies. With a median age of 63 years, 9 males and 2 females were enrolled. At baseline mean Lumbar Spine and femoral neck T-scores were -1.08 and -1.9 respectively. After bortezomib exposure the mean Lumbar Spine T-score was -0.9, and the Femoral Neck T-score was -1.5 with mean positive changes in lumbar T-score of 0.3 and at femoral neck of 0.57. Five patient’s slides from routine diagnostic core biopsy samples were digitally scanned with the Aperio XT system (Vista CA USA). The entire hematopoietic area and bony trabeculae were identified using the gene pattern recognition algorithm and each compartment was quantified. The calculated trabecular area was then divided by total surface area to obtain trabecular volume (TV). The change in mean lumbar spine bone density (0.38), and the change in mean femoral neck bone density (1.1) were statistically significant (p=0.05, p=0.04 respectively). At the same time the change in femoral trabecular volume (25.5) was also found statistically significant (p=0.02 paired t-test). This pilot study has confirmed that patients treated with single agent bortezomib experience a significant increment in bone mineral density at lumbar spine and at femoral neck even after two months of therapy and for the first time we were able to report parallel statistical changes in TV by micro-CT analysis of routine diagnostic bone marrow biopsies.
counts (p<0.05) as compared to their heterozygous and wild-type counterparts. On the other hand, heterozygous ET patients had significantly elevated (p<0.05) haematocrir levels as compared to their wild-type counterparts. JAK2 V617F positive patients had more incidence of splenomegaly and thrombosis although this was not directly related to the degree of allelic burden. However, homozygous patients with PV demonstrated a higher incidence of constitutional symptoms and required more treatment. Conclusions. Although the incidence of JAK2 V617F mutation in MPNs is similar to previous studies, there is a higher incidence of homozygosity in patients with PV in Malaysia which is associated with significantly higher leukocyte counts and lower platelet counts, higher incidence of constitutional symptoms and more requirement for treatment. Further research is required to elucidate this further.

**1343**

**JAK2 MUTATIONAL SCREENING: HIGH RESOLUTION MELTING CURVE ANALYSIS OR SEQUENCING?**

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Background. The V617F mutation in the JAK2 gene is found in 95% of the patients with Polycythemia Vera (PV) and about half the patients with Myelofibrosis (MF) and Essential Thrombocythemia (ET). Commonly used methods for the detection of the mutation includes allele specific PCR, ARMS and sequencing. More recently however, high resolution melting curve analysis is being used as a screening tool as it is rapid. The aim of the study was to use high resolution melting curve analysis to screen exon 14 of the JAK2 gene in a cohort of patients with myeloproliferative neoplasms using copy DNA as template. The presence of the mutation in both the myeloid and lymphoid lineage was also investigated. Methods: A cohort of 15 patients diagnosed with several MPNs according to WHO 2008 criteria was selected. Blood was collected and the two cell lineages were separated using density gradient centrifugation and magnetic bead selection. RNA was extracted from each cell type, converted to cDNA and used as template for high resolution melting curve analysis and sequencing. Results: The V617F mutation was identified in ten of the fifteen patients and the mutation was found in both cell types for each patient. Sequencing identified the mutation to be negative in five patients, heterozygous in nine of the patients and homozygous in one PV patient. The melting curve data did not correlate with these findings. Conclusions: In the study the V617F mutation was the only identified mutation in exon 14. The mutation was found in 83.3% of the PV patients, 60% of the ET patients and 40% of MF patients when sequencing was performed which correlates well with the current prevalence data of the mutation in literature. High resolution melting curves analysis was unsuccessful to determine the V617F mutation accurately.

**1344**

**THE MONITORING OF AUTOIMMUNE PROCESS IN PATIENTS WITH ACUTE AND CHRONIC MYELOID LEUKEMIA**

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The inadequate reaction of the immune system on autoantigens develops due to the breakdown of the mechanisms supporting immunologic tolerance, and can be observed in blood diseases. It has been established that in patients with acute myeloid leukemia, before treatment, that the maintenance of T-regulative CD4+CD25+-lymphocytes in peripheral blood has lowered an average of 3.5 times in comparison with control group (p<0.05), whereas levels of autoantibodies in double-stranded deoxyribonucleic acid (DNA) of class M (anti-dsDNA-IgM) and class G (anti-dsDNA-IgG) in blood serum has raised an average of 2.3 and 2.2 times in comparison with control parameters (p<0.05). Between the number of T-regulative lymphocytes and levels of anti-dsDNA-IgM and anti-dsDNA-IgG a strong return correlation interrelation has been found. Spearman’s coefficient of rank correlation (r) was equal to -0.92 and -0.81 accordingly. In patients with chronic myeloid leukemia in acceleration phase before beginning treatment the number of T-regulative lymphocytes in peripheral blood has lowered by 6.1 times (p<0.01), and levels of anti-dsDNA-IgM and anti-dsDNA-IgG in serum have raised an average of 3.5 times (p<0.05) and 5.3 times (p<0.01) compared to control parameters. These patients also revealed a strong return correlation between the quantity of T-regulative lymphocytes and levels of anti-dsDNA-IgM (r = -0.85) as well as anti-dsDNA-IgG (r = -0.92). Therefore, one of the probable factors promoting the breakdown of immunologic tolerance at acute myeloid leukemia and chronic myeloid leukemia, can be the significant reduction of the subpopulation of T-regulative lymphocytes.

**1345**

**EFFECCTOR AND IMMUNOREGULATORY CELL IN POLYCYTHEMIA VERA**

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Background. Polycythemia vera (PV) is a myeloproliferative disorder of slow development, which may allow the development of an immune response. It is characterized in most cases (95%) by the presence of a mutation in the JAK2 gene, which constitutive activation leads to the proliferation of myeloid cells, especially red blood cells. Aims. We have focused our work on natural killer (NK) cells in since, on the one hand, NK cells have antitumor and antiviral properties, while, on the other hand, abnormalities of these cells, including a decrease in cytotoxic activity, have been described in the majority of haemopathies. Thus, we hypothesized that, in PV, NK cells share phenotypic or functional abnormalities that allow immune escape of the malignant clone. Methods after free and informed consent 16 patients before any cytotoxic treatment (15 of them sharing the the mutated JAK2 gene) and 25 healthy controls (matched for age and sex ratio) were studied for NK phenotype and function, together with analysis of the various immune cell subpopulations. Results. While an equivalent number of lymphocytes were numbered in control subjects and in patients with polycythemia vera, we observed in the latter an increased number of NK cells. Nonetheless, these NK cells displayed poor cytolytic activity, despite normal perforin and granzyme expression. Phenotypic analysis revealed an increased expression of inhibitory receptors. Conclusions. We now have to explain the impaired cytolytic activities of NK cells by two main research axes; first, we will analyse the transcriptomic profile of normal versus polycythemia vera patients NK cells. Then, we will focus our attention on the transcriptional regulation of inhibitory and activating receptors in normal versus polycythemia vera patients NK.

**1346**

**VITAMIN D 24-HYDROXYLASE (CYP24A1) GENE IS UPREGULATED IN JAK2V617F POSITIVE ET AND PMF**

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Background: Vitamin D is myeloid cell-differentiating and proliferation controlling molecule. One of the metabolizing enzyme for vitamin D synthesis and degradation is the mitochondrial enzyme D-24-hydroxylazyme (CYP24A1) that maps to 20q13.2, below the telomeric end of common deleted region in MPN. Aim: We studied the expression of CYP24A1 in a cohort of MPN patients and associated this to their JAK2 V617F mutation status and hematological data. Methods: Fifty newly diagnosed, untreated patients with MPN (12 PV, 26 ET and 12 PMF) were taken into the study after obtaining informed consent. RNA was isolated from unfractionated bone marrow MNCs and after RT tested for expression of CYP24A1 (AB Hs00989014 Taqman Assay) in AB7300 Real-time PCR analyzer.

Data of relative expression of CYP24A1 were correlated to JAK2 mutation status and their core hematological parameters. Results: For the entire MPN group CYP 24A1 was not differently expressed concerning haematologica | 2011; 96(s2) | 543
the presence of JAK2V617F mutation: median ∆CT = 7.223 for JAK2+ vs. 8.777 for JAK2− (p<0.10). However, when ET-JAK2+ (67% ET%) and PMF-JAK2+ (66% PMF) were compared to their JAK2-mutation negative counterparts the expression was significantly higher in JAK2+ cases (7.48 vs 8.92, and 7.44 vs 8.42 respectively p< 0.05 (Figure 1)). There was no cor-
relation of CYP24A1 expression with patients' age, blood counts or ALP score. Conclusion: CYP24A1 gene for metabolizing the 1,25(OH)2D3, its relation of CYP24A1 expression with patients' age, blood counts or ALP score. Conclusion: CYP24A1 gene for metabolizing the 1,25(OH)2D3, its

determination and proliferation, this observation deserves further studies.

1JAK2 V617F MUTATION AND JAK2 46/1 HAPLOTYPE ANALYSIS IN
a GROUP OF PATIENTS WITH ISCHEMIC STROKE. A PRELIMINARY
REPORT
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Background. The JAK2 V617F mutation, the most common marker of
the myeloproliferative neoplasms (MPN), has been reported to occur also
in some non-thrombotic events, including ischemic stroke, without any sign of an overt MPN. The JAK2 46/1 haplotype is a pre-
disposing factor for the JAK2 V617F-positive MPN. Aims. To evaluate the
frequency of the JAK2 V617F mutation in a group of patients with ischemic stroke, and the possible contribution of the JAK2 46/1 haplo-
type to the occurrence of the ischemic stroke. Methods. Ninety-five
patients with ischemic stroke, without any overt MPN, and 150 individ-
uals without ischemic stroke or MPN, were included in the study. The
JAK2 V617F mutation was assessed in all the patients by a semiquanti-
tative tetra-primer PCR assay. The n10974944 (C/G) SNP, in which the
G allele tags the JAK2 46/1 haplotype, was analyzed in all the patients and
controls by a PCR-RFLP assay. Results. Only one patient (1%) was
found to harbour the JAK2 V617F mutation; his mutant allele burden
was less than 5%. The CC/CG genotypes of the JAK2 rs10974944 SNP
had a similar distribution both in patients and controls (41% versus
45.3%, p-value >0.05). Summary/conclusions. Our results indicate that
the JAK2 V617F is a rather rare finding in patients with ischemic stroke,
without overt MPN. Also, the V617F-predisposing JAK2 46/1 haplo-
type, does not seem to have a significant contribution to the occurrence
of the ischemic stroke.

1348
COMPARISON OF TWO REAL TIME PCR METHODS FOR JAK2-V617F
ALLELIC CHARGE DETERMINATION
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Background. In 2005, JAK2V617F mutation was detected in the vast
majority of patients with PV and in nearly 50% of patients diagnosed
of ET and PMF. Nowadays, several efforts have been made in order to
standardize JAK2 allelic charge quantification, which has been consid-
ered to be useful in monitoring bone marrow transplanted patients or
treated with alpha interferon or new JAK2 inhibitors. Aims. To compare
two determination methods for allelic charge of JAK2-V617F MATERI-
AL AND METHODS: 47 DNA samples of patients with a JAK2V617F
positive myeloproliferative disorder were studied by two quantitative
methods: 1) The standard one (Ipsogen Kit) was performed according to
manufacturer instructions. It uses specific primers for the mutated allele
and for JAK2 wild-type, normalizing the number of copies with mutant
plasmid curves for the mutated and wild-type alleles. 2) The second
one is also based on the use of specific primers for the mutated allele and
for the total JAK2. We used as calibration curves DNA dilutions from
HEL cellular line in K562 for the mutated allele and DNA dilutions from
a healthy donor for the total JAK2. In both cases LightCycler platform
was employed for the PCR and the ratio of mutated JAK2/total JAK2
was calculated as well. Results were analyzed by statistical methods.
RESULTS: 1) Statistical analysis by concurrent test shows a correlation
coefficient of 0.9421 (95% CI of 0.9033-0.9656). 2) Using Passing Bablok
method, the linearity test, which is used to determine how data are
adjusted to a line, shows lack of significant linearity deviation (p>0.10).

However, the line that correlates both procedures is above the line of
equality, which might slightly underestimate the quantity given by standard method, being the bias greater in medi-
un. High values are documented by Bland & Altman analysis. 3) Using T-test to compare the means of both methods, we determined
that the mean of our method is lower in an average of 3.61 (95% CI -
5.5577 to -1.6486) with a p=0.0006. This was confirmed by U-Mann
Whitney test (p=0.0001). This bias is based on the coefficient of concor-
dence between both methods is quite high. 2) There exists a bias of 5.6 units lower in our method, being greater in higher values, which indicates
that the bias is not constant, but proportional to those values. 3) Both
methods are useful for MRD study, as it is within the lowest values where
the concordance is higher.

1349
CLINICAL AND BIOLOGICAL CHARACTERISTICS ACCORDING TO
THE BURDEN OF JAK2V617F MUTATED ALLELE IN BCR-ABL NEGATIVE
MPNS
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Background. The association of JAK2V617F mutation with BCR-ABL
negative myeloproliferative neoplasms (MPNs) has been one of the most
seminal medical discoveries in recent years. Although it is a controver-
sial issue, some groups have shown that the amount of V617F allele cor-
relates with a more pronounced myeloproliferative phenotype favoring
a higher hemoglobin level and leukocyte count, higher risk of puritus,
splenomegaly and thrombosis and more probability of transformation
either to myelofibrosis or to acute myeloid leukemia. Aims. The aim of
this study was to analyze the prognostic relevance of JAK2V617F muta-
tional status and the allele burden of mutated cases in serial newly diag-
nosed patients with MPNs who had not received previous treatment.
Methods A total of 128 consecutive patients were included (median age
65; 59 males) with BCR-ABL negative classic MPNs (90 ET, 20 PV, 18
PMF) fulfilling the 2008 World Health Organization criteria. This study
was conducted in accordance with the Declaration of Helsinki. Genom-
ic DNA was extracted from peripheral blood samples using the QiAamp
DNA mini kit (Qiagen). All samples were coded and assayed blindly in
duplicate for the JAK2V617F mutation. To detect the presence or absence
of JAK2V617F mutation an Allele-Specific PCR using TaqMan allelic dis-
crimination was used. This assay is based on the simultaneous use of 2
specific TaqMan probes and the measurement of the respective fluores-
cence of the 2 alleles (FAM for V617F and VIC for wild-type) to differen-
tiate the amplification of each allele. The JAK2 MutaQuant assay (Ipsog-
gen, Luminy Biotech) was used to detect the JAK2V617F quantity in
genomic DNA by real-time detection of fluorescent signals using double-
dye hydrosyl oligonucleotide probes with calibration standards at 4 dif-
f erent concentrations, according to the manufacturer’s protocol. Labora-
tory parameters (red blood cell indexes, leukocyte and platelet counts)
and clinical data (constitutional symptoms, complications and progres-
sion) were collected. Results A total of 81 (65%) patients were JAK2V617F
positive (57.8% ET, 95% PV and 55.5% PMF). The median value of
V617F allele burden was 25.93% (range, 1.26%-95.02%). Neither a
JAK2V617F mutated status nor the quantity of burden of mutated allele
was correlated with the presence of constitutional symptoms or compli-
cations (thrombosis, hemorrhage, transformation or others). Patients
harboring a JAK2V617F mutation had significantly higher hemoglobin
level (mean (SD) 11.4 (4.7) g/dL vs. 9.9 (9.0) g/dL, p=0.021) and lower platelet
count (mean 650 (373) x109/L vs. 780 (463)x109/L, p=0.018). By consider-
ing the quantity of the JAK2V617F allele burden, a statistically significant
correlation with leukocytosis (p=0.027, Rho Spearman positive coeffi-
cient 0.252) was observed. Conversely, wild-type patients had a high-
er probability of progression (15%), than those harboring the mutation
(4%), p=0.032. Summary In newly diagnosed MPNs the presence of
JAK2V617F showed statistically significant association with a more
pronounced myeloproliferative phenotype favoring higher hemoglobin lev-
el and leukocyte count. Nevertheless the platelet count was lower in mutated cases. When considering the quantity of the JAK2V617F allele burden, statistically significance was only confirmed for leukocytosis. The risk of progression was significantly higher in wild-type patients.

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**1350 SOMATIC MPL MUTATIONS ARE NOT FOUND IN A LARGE COHORT OF PEDIATRIC PATIENTS WITH SPORADIC ESSENTIAL THROMBOCYTHEMIA**

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More than 50\% of adult but only about 25\% of pediatric patients with Essential Thrombocythemia (ET) carry JAK2V617F mutation. Furthermore, 1 to 9\% of adult ET patients carry mutations in the thrombopoietin receptor, (MPL) gene, leading to ligand-independent activation. The known somatic mutations of this gene are MPLW515L, MPLW515K, MPLW515A while germinal MPLS505N mutation was discovered in familial thrombocythemia. ET rarely occurs in pediatric patients and few is known about MPL mutations in sporadic pediatric ET. To study the prevalence of MPL mutations in children with sporadic ET, this study includes ET pediatric patients (29 females and 24 males, age range 0.6-18 years) who have been diagnosed with ET according to WHO criteria. In all cases platelet count continuously exceeding 600x10^9/L in the absence of known cause of reactive/secondary thrombocythemia. Five patients (9.4\%) resulted JAK2V617F-positive and were excluded from further analysis. We investigated the remaining 48 patients for MPL mutations with direct sequencing of granulocytes DNA. As controls, we searched MPL mutations in 46 ET JAK2V617F-negative adult patients with ET. We found only one germinal mutation (MPLS505N) in a girl coming from Rome area; her thrombocytoic mother and her grandfather, who had normal platelet count, carry the same mutation. This family seems to belong to the same cluster described by other authors (Teefil et al. Haematologica 2009). We didn’t identify any MPL somatic mutation in pediatric population but MPLW515L in 4 (9\%) adult patients. Our data confirm that pediatric thrombocythromes are heterogeneous diseases. In this rare set of patients there is a very low probability of bringing MPL mutatation; MPL mutations are even rarer.

The biological basis in pediatric ET seems different than in adults.

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**1351 THE OLFACTOMEDIN-4 GENE IS HIGHLY EXPRESSED IN PRIMARY MYELOFIBROSIS**

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**Introduction.** Olfactomedin 4 (OLF4M4) is a member of the olfactomedin-related glycoprotein family, which is specifically expressed in neutrophils and gastrointestinal tract. The findings of highly expressed OLF4M4 in immature neutrophils in the bone marrow and markedly downregulated in neutrophils in peripheral blood might suggest OLF4M4 to be involved in trafficking of neutrophils from bone marrow into peripheral blood. Being mutated in cell cycle regulated cell adhesion and inflammation we focused upon OLF4M4 as a candidate gene to be involved in these processes in PMF and related neoplasms.

**Patients and Methods.** Gene expression microarray studies have been performed on whole blood from control subjects (n = 21) and patients with ET (n =19), PV (n = 41), and PMF (n = 19). Gene expression profiles were generated using Affymetrix GeneChip HG-U133 2.0 Plus microarrays recognizing 54,675 probe sets (38,500 genes). Total RNA was purified from whole-blood and amplified to biotin-labeled aRNA and hybridized to microarray chips. Results: 20,499, 25,507, 17,417, and 25,421 probe sets were identified to be differentially expressed between controls and patients with ET, PV, PMF, and CMPNs as a whole, respectively (false discovery rate (FDR) adjusted p values < 0.05). Amongst the 50 most up-regulated genes in ET, PV and PMF, the OLF4M4 gene and several other genes encoding constitutents of neutroph granules (MMP8, DEF4A, ELA2, CRISP3, CTSG, AZU1, MPO, BPI, PRTN3, LTF) were significantly and uniquely deregulated in PMF patients, not being found among the Top-50 in ET and PV. Among these genes, the MMP8 gene displayed by far the highest upregulation (fold change (FC) 22.5 and FDR adjusted p value 6.9x10^{-10}) followed by DEF4A (FC 12 and FDR adjusted p value 9.4x10^{-10}), ELA2 (FC 12 and FDR adjusted p value 9.5x10^{-10}) and OLF4M4 (FC 11 and FDR adjusted p value 8.4x10^{-10}). Exon-borne mutations showed no significant changes in either ET or PV as compared to controls.

**Discussion and Conclusion.** Using transcriptional profiling of whole blood, we have for the first time identified a highly significant and unique upregulation of the OLF4M4 gene in patients with PMF. This gene may be involved in abnormal trafficking of immature myeloid cells into the circulation with egress of CD34 + cells from the bone marrow to extramedullary sites in spleen and liver. In support of this contention was the concomitant highly upregulated MMP8 and ELA2 genes, encoding enzymes which are considered to account for altered adhesion of myeloid progenitors to bone marrow niches in myelofibrosis. The highly upregulated OLF4M4 may also contribute to clonal expansion both by enhancing myeloproliferation and myelocoaccumulation consequent to decreased apoptosis. In conclusion, we have shown that OLF4M4 together with other constituents of neutroph granules are highly expressed in children with PMF but not in ET and PV patients.

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**1352 MOLECULAR MARKERS IN MYELOPROLIFERATIVE NEOPLASMS THE NEED OF A STUDY ALGORITHM**

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**Introduction.** The molecular markers (JAK2 and MPL mutations) have been proven to have an enormous value not only for the understanding of the MPN's etiology and pathogeny but also for diagnosis. According to recent data, the JAK2 mutations are identified in 90-95\% of the Polycythemia Vera (PV) and in 50-55\% of Essential Thrombocythemia (ET) and Primary Myelofibrosis (pMF). Objectives. Identification of JAK2 and MPL mutations in a group of 271 patients suspected of MPN. Material and Methods. Observational, retrospective and descriptive study in a group of patients suspected of MPN, studied in the Hematology Laboratory of Centro Hospitalar de Coimbra between the 1st January 2008 and 31st December 2010. JAK2 V617F mutations were studied by ASO PCR and JAK2 exon12 and MPL (exon 10) mutations were studied by SSCP and sequencing. All data collected were statistically worked using SPSS® v 17.0. Results. During the period analyzed, we studied 271 samples from patients suspected of MPN (175 from our out-patient clinic - Group 1, and 96 sent from other hospitals - Group 2). Group 1: Median age 64 years, M:F ratio 81.94; 77/175 (44\%) studied for polyglobuluy, 98/175 (56\%) for thrombocythemia; polyglobuluy group: - 67.2\% JAK2V617F positive; 2.6\% exon12 mutations - Median full blood count (FBC) values: - (JAK2 positive) Hemoglobin(Hb) 18.5g/dL, hematocrit (Ht) 56.3\%, leucocyte (Leuk) 11.2 10^3/L and platelets 532 10^3/L; - (JAK2 negative) Hb 17.2g/dL, Ht 51.4\%, Leuk 6.4 10^3/L and platelets 211 10^3/L. Thrombocytosis group - 54\% JAK2 V617F positive; 2.8\% with MPL (exon 10) mutations. Group 2. In this group, as we only received blood samples (BS) for the JAK2 V617F screening, we could only analyze the information that was sent along with the samples. Median age 66\%, M:F ratio 60:36; 46 (47.92\%) studied for polyglobuluy, 50 (52.08\%) for thrombocytosis; Polyglobuluy group - 26.09\% JAK2 V617F positive; - Median FBC values: Hb 17.2g/dL, Ht 52.4\%, leuk 7.07 10^3/L and platelets 228 10^3/L. Thrombocytosis group - 44\% JAK2 V617F positive Conclusions. In group 1 - patients observed in our department - we diagnosed: 54 PVs, (51 JAK2 V617F positive, 2 exon12 positive and 1 PV JAK2 negative), corresponding to 98.15\% JAK2 positive PVs; 72 ET (61,11\% JAK2 V617F positive and 2.78\% MPL exon 10 positive); 10 pMF (70.0\% JAK2 V617F positive). Among all the MPN diagnosed we were able to identify a molecular marker in 75.71\% of the patients. In Group 2, BS sent to our lab, curiously the median FBC values were similar to the JAK2 negative polyglobuluy from group 1. Even though we lacked clinical information in group 2, when we compared the percentage of JAK2 V617F positivity in both thrombocythemia groups (1 and 2), the results were similar (54\% Vs 44\%). When comparing the polyglobuluy groups, we found a very different positivity of JAK2 V617F: 67.23\% group1 Vs 26.09\% group 2. This last data emphasizes the importance of a study algorithm for polycythemia vera which are, undoubtedly, a diagnostic challenge.
RBC transfusion-dependency, increase Hb concentration and decrease respectively. The count of platelets before and after EPO-treatment was notations. We observe no one case of thrombosis during the period >6 months. The median age of patients was 70 years (range 50-84). All patients had anemia with initial Hb concentration 5.3-9.9 g/dl. RBC transfusion-dependency was defined as the persistence of RBC transfusions. In whole group of PMF patients with anemia (n=26) pos- sions shown to be associated with the pathogenesis of Philadelphia chro- mosome - negative myeloproliferative neoplasms (MPNs). Various molecular approaches have been applied, yet universally accepted methods have been limited because of problems of specificity to direct sequencing and had a much higher sensitivity of 1% mutant alleles.

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STUDY REGARDING THE ASSOCIATION BETWEEN MULTIPLE PRIMARY CANCERS THAT INCLUDES A MYELOPROLIFERATIVE DISORDER AND THE METABOLIC SYNDROME

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ANAGRELIDE? THE FOX (FRANCE OBSERVATOIRE XAGRID) STUDY

ESSENTIAL THROMBOCYTHEMIA AND FAILURE OF FIRST LINE TREATMENT: WHAT IS THE OPTIMUM METHOD FOR INITIATING ANAGRELIDE? THE FOX (FRANCE OBSERVATOIRE XAGRID) STUDY

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Background. Essential thrombocythemia (ET) is an acquired myeloproliferative disorder. Studies are needed to investigate the mechanisms that explain the relationship between metabolic syndrome and multiple primary malignancies.

Aims. A prospective single-centre study of immunophenotypic and cytogenetic characteristics as well as the clinical course and therapy outcomes in consecutive patients with acute leukemia supervening on the Ph-negative MPNs. Twenty patients (pts) were studied (med. age, 61, range 34-73 years). Thirteen pts had PMF, four ET, and three PV. The type of acute leukemia was confirmed using flow cytometric methods and Immunohistochemistry. Cytogenetic analyses were performed in all patients at diagnosis of a primary disease and at the time of acute transformation of MPN. Results: The median duration of a chronic phase of MPN was 45.4 months (range 10.5-300). One pt developed acute myeloblastic leukemia (AML) M-1 (by FAB Group Classification), fifteen pts AML M-2, two pts AML M-4, and one pt AML M-7. One pt acquired the BCR/ABL-negative B-cell acute lymphoblastic leukemia. The karyotypic evolution was observed in 7/20 pts. A 48,XY,+6,+16 was observed in two pts, del(20) in two pts, and i(17)(q10); der(20) and multiple karyotypic abnormalities in one patient each. Five out of eight JAK2-V617F-positive pts lost the signal of this mutation in the leukemic phase. Ten pts had the signal of the BCR/ABL fusion. Two pts survived in CR and died in relapse. One patient in partial remission survived an average of 16.5 months. Four pts with stable leukemia were in complete remission (CR) for 59 and 50 months each. Two pts survived palliative chemotherapy or support only. Two pts are presently alive and well since the start of the treatment.

Background. BCR/ABL (Ph)-negative myeloproliferative neoplasms (Ph-negative MPN) embody polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These are clonal hematopoietic disorders and which have several clinical, laboratory and histological features in common. The discovery of JAK2-V617F and JAK2 exon 12 mutations and, more recently, mutations in MPL, N-WT1 and the BCR/ABL-negative B-cell acute lymphoblastic leukemia. The karyotypic evolution was observed in 7/20 pts. A 48,XY,+6,+16 was observed in two pts, del(20) in two pts, and i(17)(q10); der(20) and multiple karyotypic abnormalities in one patient each. Five out of eight JAK2-V617F-positive pts lost the signal of this mutation in the leukemic phase. Ten pts received intensified induction chemotherapy. Another ten were treated by palliative chemotherapy or support only. Two pts are presently alive and well since the start of the treatment.

Aims. A prospective single-centre study of immunophenotypic and cytogenetic characteristics as well as the clinical course and therapy outcomes in consecutive patients with acute leukemia supervening on the Ph-negative MPNs. Twenty patients (pts) were studied (med. age, 61, range 34-73 years). Thirteen pts had PMF, four ET, and three PV. The type of acute leukemia was confirmed using flow cytometric methods and Immunohistochemistry. Cytogenetic analyses were performed in all patients at diagnosis of a primary disease and at the time of acute transformation of MPN. Results: The median duration of a chronic phase of MPN was 45.4 months (range 10.5-300). One pt developed acute myeloblastic leukemia (AML) M-1 (by FAB Group Classification), fifteen pts AML M-2, two pts AML M-4, and one pt AML M-7. One pt acquired the BCR/ABL-negative B-cell acute lymphoblastic leukemia. The karyotypic evolution was observed in 7/20 pts. A 48,XY,+6,+16 was observed in two pts, del(20) in two pts, and i(17)(q10); der(20) and multiple karyotypic abnormalities in one patient each. Five out of eight JAK2-V617F-positive pts lost the signal of this mutation in the leukemic phase. Ten pts received intensified induction chemotherapy. Another ten were treated by palliative chemotherapy or support only. Two pts are presently alive and well since the start of the treatment.

Leukemic transformation of the Philadelphia (BCR/ABL)-negative myeloproliferative neoplasms (Ph-negative MPN) embody polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). These are clonally related hematopoietic disorders and which have several clinical, laboratory and histological features in common. The discovery of JAK2-V617F and JAK2 exon 12 mutations and, more recently, mutations in MPL, N-WT1 and the BCR/ABL-negative B-cell acute lymphoblastic leukemia. The karyotypic evolution was observed in 7/20 pts. A 48,XY,+6,+16 was observed in two pts, del(20) in two pts, and i(17)(q10); der(20) and multiple karyotypic abnormalities in one patient each. Five out of eight JAK2-V617F-positive pts lost the signal of this mutation in the leukemic phase. Ten pts received intensified induction chemotherapy. Another ten were treated by palliative chemotherapy or support only. Two pts are presently alive and well since the start of the treatment.

The aims of the study are to determine whether the switching strategy may influence the tolerability and the efficacy of anagrelide in the second-line setting, or the care of the patients. The FOX study also offers the opportunity to identify the most efficient initiation protocol for anagrelide. Patients and Methods. FOX is a multicenter, prospective, observational study assessing adult patients with ET who switch to anagrelide following failure of, or intolerance to, HU. Patients will be assessed prospectively over a 6-month period following the introduction of anagrelide treatment. During the assessment period, all relevant data available from the patients’ medical records will be compiled. No clinical examinations or procedures will be required during the study. The target recruitment is 180 patients from 60 participating centers, with the aim of obtaining 160 evaluable cases. Each investigator will enrol each consecutive patient fulfilling the inclusion criteria. The study analysis will compare the number of patients maintaining treatment with anagrelide after 6 months, including details of the initiation schedules, using a Fisher exact-test. The study is ongoing and final results are expected in June 2012. The study is sponsored by Shire Pharmaceutical Development Ltd. Summary/conclusions. ET is a chronic condition with limited treatment options that requires long-term therapeutic management. Where there is intolerance or lack of efficacy with the first line treatment, HU, anagrelide is indicated as second-line therapy. The method of initiating treatment switch to anagrelide is not universally agreed but may have an impact on its efficacy and tolerability. This issue is currently being assessed in the FOX study in order to determine the optimal procedure for initiating treatment with anagrelide.
HIGHLY SENSITIVE QPCR INCREASES THE RATE OF DETECTION OF JAK2-V617F IN PATIENTS WITH MYELOPROLIFERATIVE NEOPLASMS

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Background. Approximately 95% of patients with polycythemia vera (PV) and 50% of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF) harbour the JAK2-V617F mutation with smaller numbers exhibiting either MPL or JAK2 Exon 12 mutations. The presence of JAK2-V617F has become a major diagnostic criterion for these disorders, emphasising the need for accurate testing. Recent data from external quality assessment schemes involving large numbers of European laboratories suggest marked variation in performance of assays measuring JAK2-V617F allele load, yielding highly divergent test results. Targeted therapy using JAK2 inhibitors within clinical trials, and other modalities such as transplantation, may require accurate molecular monitoring. Interestingly, patients testing negative for JAK2-V617F may respond to targeted therapy and are not distinguishable from those with mutant JAK2. This is consistent with the finding of activated JAK signalling, but could also suggest that they may harbour the mutation at a lower level than presently detectable.

Aims. To reassess a cohort of patients with myeloproliferative neoplasms (MPN), negative for JAK2-V617F by standard allele-specific PCR (AS-PCR), using highly sensitive real-time quantitative PCR (qPCR). Methods. We identified patients from our hospital laboratory records who had tested negative for JAK2-V617F mutation by AS-PCR between 01.08.2005 and 01.08.2010. AS-PCR was performed using a common reverse primer and two forward primers. The first forward primer was specific for the mutant allele, with an intentional mismatch at the third nucleotide from the 3' end, giving a 205-bp product. The second primer amplified mutant and wild type alleles to give a 364-bp product, acting as an internal control. During the study period, 824 samples had been tested for JAK2-V617F mutation, of which 569 tests were reported as negative. Of patients testing negative, 84 met the WHO criteria for ET, 23 were diagnosed as PMF, 6 as PV, 4 as myeloproliferative/myelodysplastic overlap (MPN/MDS) and 11 as MPN, unclassifiable (MPN-U). Peripheral blood samples were analysed using a qPCR assay which has been shown in QC rounds conducted within the European LeukemiaNet to detect a 0.1% level of JAK2-V617F. V617F was quantified relative to wild-type JAK2, and to albumin, used as an independent control gene. Results. 47 samples were tested, 52 ET, 12 MF, 1 PV, 1 MPN/MDS and 1 with MPN-U. In 46 cases, the albumin gene was amplified with a mean cycle threshold (CT) of 22 cycles (range=20-25) but no V617F was detected. One patient, with PMF, had 5.2% JAK2-V617F relative to wild-type JAK2 and albumin. This patient has since undergone a reduced intensity conditioning allogeneic stem cell transplant. At reassessment four months post transplant, with full donor chimaerism, the V617F level had fallen to 0.2%. Summary/conclusions. In this small cohort of 47 patients, previously diagnosed as having JAK2 negative MPN, one was found to have a significant V617F mutant burden relative to wild type. There was a significant reduction in mutant load at reassessment four months post transplant. This finding supports qPCR as a high sensitivity method to detect JAK2-V617F and its potential role in monitoring residual disease during treatment.

MYELOPROLIFERATIVE DISEASES AS A CAUSE OF THROMBOSIS

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Myeloproliferative diseases (MPD) belong to the group of clonal malignant diseases of parent cell hematopoiesis, characterized by abnormal increase of one or several blood lines with normal or nearly normal maturing of those cells, both in bone marrow and in extramedullary hematopoietic organs. According to the data from literature, thrombotic complications occur in about one third of patients with PV. The main cause of morbidity and mortality in ET is bleeding and thromboses. About 49% of patients showed a tendency to thromboses, and 60% to bleeding. Thrombotic complications are, as a rule, arterial thromboses, although venous thromboses appear as well, particularly in the deep veins of lower extremities. The most common sites of arterial thrombosis are cerebrovascular, peripheral, vascular and coronary circulation. The patients are commonly young, healthy, postmenopausal women, with excessive blood vessels. The aim of the paper is to show the extent to which the thrombotic complications occur in patients with certain forms of chronic myeloproliferative diseases. The investigation included 219 subjects of both sexes, between 17 and 83 years of age with the diagnosis of MPD. The patients were divided into five groups: A. Chronic myeloid leukemia (CML)-group; B. Polycythemia vera (PV)-group; C. Idiopathic myelofibrosis (IMF)-group; D. Essential thrombocythemia (ET)-group; E. Myeloproliferative disease that cannot be classified (MPS)-group. The methods of clinical examinations, endoscopies, exonsographies and computer-assisted tomographies have been used. In our research prevalence of thrombotic complication was 20% of all subjects with MPD. In the group with CML about 5%, in the group with PV about 30%, and in the group with IMF about 7,6%, in the group with ET about 63% and in the group with MPS about 42% of patients had one or more thrombotic complications. The highest percentage of thrombotic complications is within the group of subjects with PV, that is statistically more significant compared to PV (p<0,05), IMF and CML (p<0,001). We proved that prevalence of thrombosis is in a statistical dependence on the type of MPD. Mechanism of thrombophilic state in patients with MPD is not entirely clear. Uncorrected polycythemia with elevated values of hematocrit and increased blood viscosity, increases the tendency to thromboising. Impaired platelet function within MPD could also be significant for the development of thrombotic and hemorrhagic complications as well. Some authors have described the disturbances in the fibrinolytic system and the function of natural coagulation inhibitors (AT III and protein C),

MYELOPROLIFERATIVE NEOPLASMS, STEM CELL MOBILIZATION AND AUTOLOGOUS TRANSPLANTATION

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Background. Myeloproliferative neoplasms (MPN) are chronic disorders that evolved for many years but they can be complicated by thrombotic complications or haematological transformations as myelofibrosis or acute leukaemia. Actual management of MPN diminishes thrombotic complications. Stem cell collection and autografting should be used in this setting in patients with both haematological malignancies. Aims - We report two cases of successful stem cell mobilization and autologous transplantation for plasma cell malignancies with concomitant myeloproliferative neoplasms, the first with multiple plasmacytoma and essential thrombocytopenia and the second with multiple myeloma and primitive polycythemia. Methods - In our two cases, the myeloproliferative neoplasm was diagnosed several years before plasma cell malignancies (one patient ten years respectively). Both patients were treated with hydroxyurea for ten and nine years before stem cell collection. Symptomatic plasma cell malignancies was then diagnosed in both patients and treated with bortezomib dexamethasone cycles. Stem-cell mobilization was realized after granulocyte-colony-stimulating factor and allowing 7 x 106 and 5 x 106 CD34+ cells/kg, respectively. Conditioning chemotherapy before autografting was melphalan 200 mg/m2. No complications were noted during all these procedures. Platelet and granulocyte recovery after autografting were respectively 19 and 16 days for the first patient and 14 and 12 days for the second. Results - Our two cases show the feasibility of stem cell mobilization and autologous transplantation for haematological malignancy with concomitant myeloproliferative neoplasms. Although disease evolution of myeloproliferative neoplasms has reached the first decade for our two patients, stem cell mobilization seems to be possible. Moreover, cytoreductive treatment as hydroxyurea and possible fibres seem do not influences stem cell mobilization. However, autografting does not seem to influence the outcome of MPN as the platelet count returned to normal range six months after autografting for our first patient. Conclusions - Stem cell mobilization and autologous transplantation are feasible for patients with haematological malignancy and concomitant myeloproliferative neoplasms, even after decades of history. It was an important because a increased risk of lymphoid neoplasms has been described for patients with MPN compared with general population.
which further contribute to the tendency of thrombosizing in patients with MPD. One of the explanations for increased risk of thrombosis in patients with ET is that the total amount of thrombin generated on the surface of platelets in those patients, has been significantly higher than with the control group or in patients with reactive thrombocytosis. Thrombotic complications often accompany chronic myeloproliferative diseases. They are usually present in patients with ET and also in patients with PV and MPD and much less frequently in patients with CML and IM, which is in accordance with data from literature.

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ACTUAL MANAGEMENT OF PH NEGATIVE MYELOPROLIFERATIVE MALIGNANCIES - THE EXPERIENCE OF AN EST EUROPEAN CLINIC
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Background. The description of the JAK2V617F mutation in classical Philadelphia (Ph)-negative MPN provide us a rational target for novel innovative treatment strategies but currently, clinical studies testing various JAK2-inhibitors in PV, ET as well as in primary and secondary myelofibrosis (MF) are under way. Interestingly, first data indicate that despite interferon α activity in platelet size reduction and improvement of constitutional symptoms, these inhibitors might not sufficiently reduce disease burden. So we tried to compare efficacy of well established treatment strategies, such as inhibition of thromboctye aggregation by low dose aspirin, cytotoxics (e.g. hydroxyurea, anagrelidum), immuno- and stroma-modifying therapy with interferon, tyrosine kinase inhibitors and, in selected cases, allogenic stem cell transplantation and to make a correlation between the response at these therapeutical alternatives and the mutational status. Methods and results: We investigated a lot of 430 patients diagnosed in our clinic between 2004 and 2010; the study population was female in proportion of 55%, ages ranged from 22 to 72 years (median age 52). We tried to establish the indications on our study group for cytoterapeutic therapy in which the key targets were to reduce thrombohemorrhagic complications, relieve disease-related symptoms and minimize the risk of transformation to secondary myeloid malignancy such as myelodysplasia, leukemia, and secondary myelofibrosis. We correlate the rate of disease progression with the mutational status and investigated the role of interferon treatment in JAK2 V617F positive cases. We tried to establish if there is a significant molecular response in these subset of patients. In our study 87% of patients treated with IFN had a hematologic response, and from these 71% were complete responses (CRs). Conclusions: We reported that in our study group the patients with myeloproliferative disorders treated with IFN-2b had lower JAK2-V617F allele burdens compared with a control group that did not receive IFN therapy. The JAK2-V617F was present in 71/106 (66.98%) patients with MPNs or in 36/41 with PV (87.80%), 25/43 with ET (58.14%), 5/9 with PMF (55.56%) and 5/13 MPNs-unclassified (38.46%) disorder. The JAK2-V617F mutation was absent in patients with secondary erythrocytosis and secondary thrombocytosis. There were significant differences in hemoglobin and hematocrit level, leukocyte alkaline phosphatase score and white blood cells at diagnosis in JAK2-V617F non mutated versus mutated patients with MPNs. Vascular events were present in 23/106 (21.7%) patients with MPNs or more specifically in 13/41 (31.7%) with PV, 5/40 (12.5%) with ET, 1/9 (11.1%) with CML, and 1/3 (7.7%) with MF. The majority of those patients with one or more vascular events were JAK2-V617F positive. When analyzed within each entity it could be emphasized that one or more vascular events, or recurrent thrombosis was detected in 13/41 (31.7%), 7/41 (17.1%) cases, respectively with PV, or in 10/15 with JAK2-V617F positive status. In patients with ET, compared to PV vascular events (8/43) were less present (18.6%). Though, as noticed for PV, most of those patients (6/8) were associated with JAK2-V617F mutation. In patients with PMF only 1 vascular event was confirmed (patient was JAK2V617F negative), as well as in patients with MPN-u (patient was JAK2 positive).
Conclusions. The JAK2-V617F mutation was frequently detected in our patients with MPNs in accordance with literature data, and therefore should be incorporated in the diagnostic evaluation of patients with suspected MPNs. Further analysis should focus on contribution of the JAK2-V617F mutation in the clinical phenotype of patients with distinct subgroups of MPNs.

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PREVALENCE OF THE JAK2 V617F MUTATION IN CROATIAN PATIENTS WITH CLASSIC PH-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS
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Background. The classic Ph-negative myeloproliferative neoplasms (MPNs) are clonal disorders of multipotent haematopoietic progenitors. The Janus-associated Kinase-2 mutation JAK2 V617F in such neoplasms has been described as a frequent genetic event in majority of these patients. Aims: To investigate the status of the JAK2-V617F mutation in patients with classic Ph-negative MPNs treated in Clinical Hospital Center Rijeka and to compare it with hemoglobin and hematocrit level, white blood cells and platelet count, splenomegaly, leukocyte alkaline phosphatase score and clinical features. Methods: DNA was isolated from peripheral blood granulocytes in 115 patients: 41 with polycythemia vera (PV), 43 with essential thrombocythemia (ET), and 31 with primary myelofibrosis (PMF). JAK2V617F was analyzed in 12/14 patients who had BCs. Every patient underwent bone marrow biopsy: polycythemia vera/PMF 4 patients, essential thrombocytosis/ET 7 patients, primary myelofibrosis/PMF 3 patients. JAK2V617F was analyzed in 12/14 patients and was positive in 100%. Inherited thrombophilia was not associated with BCs. Every patient underwent bone marrow biopsy: polycythemia vera/ET 4 patients, essential thrombocytosis/ET 7 patients, primary myelofibrosis/PMF 3 patients. JAK2V617F was analyzed in 12/14 patients and was positive in 100%. Inherited thrombophilia was not found. Acquired thrombophilia was mentioned in two patients. A woman with Budd-Chiari syndrome (BCs) who was provided oral contraceptive pills, and a man with portal vein thrombosis (PVT) post- splenectomy. Patients with BCs had mean age 43.2 years (range, 35-56) and 60% were female. Three were diagnosed with PV, 1 ET and 1 PMF. One patient died after 17 years and one was scheduled for liver transplantation after 6 years. The other three patients had no signs of ascites or portal hypertension in a six-year follow up. Patients with PVT had mean age 54.8 years (range, 21-78) and 67% were male. Six were diagnosed with ET, 2 PMF and 1 PV. 2 patients died and 1 with portal vein thrombosis. Nobody died. All of the patients have signs of portal hypertension. Mean time of follow up is 1.8 years (range, 0.2-6). All patients were managed with routine anticoagulation therapy from diagnosis. Three patients had indications for decompressive procedures such as TIPS, all in the group of BCs. Summary/Conclusions: JAK2-V617F mutation is frequent presenting complication of undiagnosed MPNs. In patients with PV, ET, portal hypertension is a virtually constant feature. The resulting hypersplenism and hemodilution decrease the accuracy of blood cell counts for MPD diagnosis. The
atypical peripheral blood picture in the setting of SVT has led to a vari-
yety of neoplasms such as *latent* MPDs. In our study, all patients with
MPD and SVT were positive for the mutation JAK2V617F. The presence
of this mutation may predict a more aggressive phenotype with an
increased risk of thrombosis.

### 1366 ESSENTIAL THROMBOCYTHEMIA: CORRELATION BETWEEN JAK2 ALLELE BURDEN AND CLINICAL OUTCOME

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Essential thrombocythemia (ET) is a myeloproliferative disorder
characterized by persistently elevated platelet count (>450×10^9/L, 2008 WHO
classification) with normal red cell mass. Thrombosis, acute myeloid
leukemia or myelofibrosis are its main complications. Janus kinase 2
mutation (JAK2V617F) appears involved in the molecular etiology of
disease development. Its identification markedly simplified the approach
to clinical diagnosis and provided molecular targets for the development
of biologically targeted drugs. Recent studies reported an association
between JAK2V617F and thrombosis in patients with myeloproliferative
disorders, but it remains unknown whether inherited thrombophilia
has an impact as an additive risk factor in mutated subjects. Therapeu-
tic strategies are hydroxyurea (HU), anagrelide and alpha-interferon
(IFN), aimed to reduce platelet count and ASA to control the thrombot-
amic risk. We studied 52 patients with TE aiming to 1. evaluate variation
in JAK2V617F mutant allele burden during different treatments (HU,
anagrelide, interferon or none), 2. evaluate relationship between allele
burden and thrombosis risk factors. Evaluation of patients' thrombotic
risk factors are the DNA sequencing of KIT gene, did not show D816V mutation, but
substitution on the nucleotide 1851 codifying for a silent Val617Val
mutation. DNA sequencing did not confirm the G>T substitution on the
exon 15 that bring to a serine 715 deletion. Systemic Mastocytosis
was diagnosed in 61 years (0.4-93 years) with a mean length of follow-up of 7.7
years (0.5-44 years). 90% of patients were treated with hydroxyurea
(HU) at some stage during this follow-up, with a mean dose of
707mg/day and an average length of exposure of 4.2 years. 54 patients
were treated with non-HU cytoreduction including interferon, anagre-
lide, busulphan and radioactive phosphorous. 29 patients (15%) had
platelet counts that varied by over 100×10^9/L over a 6 week period while
on a stable dose of cytoreductive therapy. Platelet cycling occurred in all
MPN subgroups (hitherto the literature has documented this in PV only);
there was no difference in incidence according to gender or JAK2 posi-
tivity. 25 patients (6%) were taking HU at a mean dose of 920mg/day.
The mean maximum difference between the lowest and highest platelet
count over the 6 weeks was 350×10^9/L (100 - 1346×10^9/L). 60 patients
(32%) had non-melanoma skin cancers, 29 (48%) of whom had multi-
ple skin cancers. Those with skin cancers had a longer exposure to HU
than the overall population (5.7yrs vs 4.2 yrs). Those with multiple
NMSC were more predominant in JAK2V617F positive than the overall
population (5.7yrs vs 4.2 years). Those with multiple
NMSC had leg ulcers and eosinophilia (4%) experience lower HU
(79yrs). Acquired VWD occurred in 17 patients (9%), was mostly type 2
and was not associated with any haemorrhagic complications. HU
‘allergy’ was seen in 12 patients (7% of those taking HU) and predom-
nantly presented as fevers (9 patients) that recurred in all those re-chal-
 lenged with HU. Of those taking HU, 6 patients (5%) had leg ulcers and
eosinophilia (4%) and 2 patients (1%) had a thrombotic event with the majority of these (67%) occurring prior to or at
diagnosis. Conclusions. Cyclical thrombocytosis is not infrequent in
all Philadelphia-negative MPNs and appears associated with a higher
HU dose. Other features that are intrinsic to the disease (such as acquired
VWD) or occur as a consequence of therapy (skin cancers and HU-fever)
are also not uncommon.

### 1368 FIRST CASE OF KIT SER715DEL AND JAK2 VAL617VAL MUTATION IN SYSTEMIC MACROCYSTOSIS WITH ASSOCIATED ESSENTIAL THROMBOCYTHEMIA

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In March 2009 a 22-years-old woman presented to our Institution
with persistent high platelet count (>650×10^9/L) and no other peripheral
blood alterations. At physical examination splenomegaly was present;
headache, erythromelalgia and diarrhea were referred. Blood chemistry
was normal and reactive thrombocytosis was excluded. The morpho-
logical examination of the bone marrow (BM) aspirate revealed an
increase of enlarged megakaryocytes (MK) with emperipolesis phenomen.
BM smears showed also spindle shaped mast cells (MC) with oval
and eccentric nuclei and focal granule accumulation. Eosinophil forms
were increased. Biopsy specimens were hypercellular and showed a
marked proliferation of giant MK, preferentially grouped into clusters;
in addition to this myeloproliferative pattern, nodules of ovoid or spin-
dle shaped MC were present, surrounded by areas of localized dense
network of reticulin fibers. MC identity was confirmed by toluidine-blue
eosin staining; immunohistochemical staining for anti-CD117 (KIT) and CD2.
CD2 expression analysis revealed a specific immunophenotypic analysis of MC
was not performed at this time. The allele-specific-PCR demonstrated the presence of the JAK2V617F
mutation. DNA sequencing did not confirm the G>T substitution on the
base 1849 codifying for the Val617F mutation, but we detected a G>T
substitution on the nucleotide 1851 codifying for a silent Val617Val
mutation. FP11.1-PCGRA and BCR-ABL transcripts were nega-
tive. DNA sequencing of KIT gene, did not show D816V mutation,
but detected a three nucleotides deletion (ACC; bases: 2143-2144-2145),
on the exon 15 that bring to a serine 715 deletion. Systemic Mastocytosis
(SM) clinical staging showed not skin involvement, normal bone densi-
 ty and a spleen volume of 500 mL by ultrasound; serum tryptase was
17.6 ng/mL. We concluded for SM with Associated clonal Hematologi-
 cal Non-Mast cell Lineage Disease (SM-AHNMID), id est Essential
Thrombocytopenia. Because of symptoms we began treatment with IFN-
α-2a at 3 MU five times a week. The therapy was well tolerated, all
Symptoms disappeared and the platelet count was lowered to less than 350 x 10^9/L. After four months of therapy, IFN-α dose was tapered to three times a week. The BM aspirate was repeated after eleven months, showed the absence of MC and a MK reduction, while the BM biopsy demonstrated persistence of MC nodules. The specific immunocytofluorometric assay was negative. The molecular analyses confirmed the Ser715 del on KIT gene but the JAK2V617F silent mutation was undetectable. The tryptase serum level was 20.9 ng/mL. The spleen volume was 290 mL and resulted reduced compared to the baseline. This is the first case of a SM-AHNMD harboring a silent mutation on JAK2 and Ser 715 del on KIT. This KIT deletion has been previously described in GIST and if it is an activating mutation or a gene polymorphism is still matter of debate. The significance of this mutation for myeloproliferative disease is unknown. However this mutation leads to deletion of a polar amino acid placed near Tyr719 which is necessary for KIT PTK binding and downstream; this mutation could induce abnormalities in KIT local folding and signaling and contribute to pathogenesis of both MC and MK disease.

**1369**
HYDROXYUREA PLUS ANAGRELIDE COMBINED THERAPY IS MORE SUITABLE THAN SEQUENCE SCHEDULE OF THE TWO DRUGS

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Background. Hydroxyurea (HU) and anagrelide are widely used treatment of Essential Thrombocythemia (ET). Given their different mechanisms of action, they can be used as combined therapy at reduced doses. This association could represent a second-line treatment in patients considered resistant or intolerant to HU, or a therapeutic option to reduce both HU and anagrelide related side-effects. The combined use of these two drugs gives a more balanced control of the hematological parameters, improving the platelet lowering action and better controlling WBC count, known to be correlated with thrombotic risk. We avoid larger doses of either drug because anagrelide is ineffective in lowering WBC count, and higher doses of HU have an unwanted Hb lowering action.

Aims. We describe a combined sequence HU plus anagrelide therapy to improve patients’ adherence to therapy and quality of life. Patients and methods. Five ET patients received a combined sequence therapy of HU and anagrelide. They were a spontaneously formed cohort of patients treated with other medications for comorbidities. They casually requested that the daily numbers of pills be reduced. We used a sequence therapy based on the following schedule: HU at a dose of 1 g daily for 10 days, followed by anagrelide at a dose of 1 mg daily for 10 days for three months. ET diagnosis was based on the 2008 WHO criteria. Patients' mean age was 48.6 years: 3 females and 2 males. Three were JAK2V617F positive. Results. All patients received HU as first line treatment and all achieved complete hematological response (CHR); this resistance was dealt with administering a schedule of HU and anagrelide combined, at a daily dose respectively of 1 g and 1 mg, for a total amount of 4 daily pills. All patients achieved CHR. Mean platelet counts compared to the one while on monotherapy, were 673.3 vs 379.4 x 10^9/L (p=0.0003, fig.1.1vs2). Given the patients’ need to take other pills we requested that the daily numbers of pills be reduced. We used a sequence schedule and returned to the previous combination therapy. Patients achieved CHR, which had never been reached with the previous monotherapy treatment. The sequence therapy, instead, was not as effectively as the combination therapy. Furthermore, patients’ mean platelet counts were similar to those during monotherapy (673.3 vs 626 x 10^9/L; p=0.47), demonstrating the loss of CHR (fig.1.1vs3). Despite the availability of some management recommendation and guidelines on ET treatment, therapy based on the association of HU and anagrelide has not yet been codified.

**1370**
CHROMOSOMAL TRANSLOCATION T(15:17)(Q22;Q25) WITH PML-RARA POSITIVITY IN PATIENT WITH PRIMARY MYELOFIBROSIS (PMF)

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Background. Primary myelofibrosis (PMF) is a clonal stem cell disorders characterized by splenomegaly, fibrotic bone marrow, extramedullary hematopoiesis and leukoerythroblastic blood smear with dacryocytes. Approximately, one third of patients with PMF have cytogenetic abnormalities at diagnosis. They are respected as favorable (isolated abnormalities like 13q-, 20q- or +8) but also unfavorable (all others). Aims: We are presenting a male patient with PMF and with unusual cytogenetic abnormalities, translocation t(15;17)(q22;q25) and JAK-2 V617F mutation. Patient and methods: A 58-year old man was diagnosed with a six months history of abdominal fullness, weakness, weight loss and pain in left upper abdomen. In August 2009, he was referred for further diagnostic work-up to Clinics of Hematology CCS, Belgrade. The diagnosis of PMF was confirmed by bone marrow biopsy and further clonality assays were performed by karyotyping and molecular biology. PCR was applied for detection of JAK-2 V617F mutation and BCR-ABL transcripts. Afterwards, RNA was extracted from the bone marrow sample and RT-PCR has been performed according to BIOMED-1 protocol in order to analyze PML/RARα fusion transcript. Results: Physical examination revealed splenomegaly, +10cm below LCM, or 22 cm on CT scan. Patient had normal hemoglobin, 142 g/L, slight leukocytosis 18.9 x 10^9/L with mild shift to the left and normal platelet count, 279 x 10^9/L. Standard blood chemistry tests were within normal range. Core bone marrow biopsy corresponded to PMF (clusters of hyperlobulated megakaryocytes and grade 3 fibrosis). Cytogenetics from bone marrow revealed balanced translocation t(15;17)(q22;q25) in 15 metaphases and 3 metaphases with addition on chromosome 18, add(18)(p11). According to International Prognostic Scoring System patient was low risk. PCR detected bcr1
fusión transcript of PML/RARα. This result was subsequently confirmed by direct sequencing of PML/RARA gene. JAK-2 V617F mutation was also positive. BCR-ABL rearrangement was absent. Patient commenced hydroxyurea 1g/d and after 16 months he reduced splenic to 16 cm (CT) and normalized blood counts, without any signs of acute leukemia during follow-up. Conclusions: The occurrence of PML-RARA and JAK-2 V617F mutation simultaneously in the same patient is exceptionally rare event. Even though the patient was prepossessed for adverse cytogenetic profile with JAK-2 mutation indicating adverse predictors for survival, he had a good response with hydroxyurea treatment, and no evolution into promyelo-cytic leukemia.

1371 SYSTEMIC MASTOCYTOSIS WITHOUT CKIT D816V MUTATION TREATED WITH DASATINIB: A CASE REPORT
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Background: Systemic mastocytosis (SM), often termed systemic mast cell disease (SMCD), is an hematologic clonal disorder characterized by abnormal growth and accumulation of Mast Cell (MC) in one or more organs. MCs, overproduced, is a myeloproliferative neoplasm (MPN) in the 2008 revision of the WHO classification of myeloid neoplasms. The clinical symptoms and signs of SM are due to the accumulation of clonally derived mast cells in different organs, including bone marrow, skin, gastrointestinal tract, liver and spleen. In more than 80% of the patients, the cKIT mutation D816V is detectable. Those cases without the cKIT D816V mutation are considered as Systemic mastocytosis negative for D816V (SMCN). We report a case of a SM without the cKIT D816V mutation, that achieved a good response to dasatinib after failing previous imatinib therapy. 2. Case report: A 77 year old man was admitted to our hospital because of asthenia, hyporexia and fever during the three previous months. Blood analysis showed anemia, leucocytosis with monocytosis, thrombocytopenia, anemia, hyporexia and fever during the three previous months. Blood analy-

1372 BUSULFAN: STILL A USEFUL THERAPY FOR ESSENTIAL THROMBOCYTHAEMIA (ET) AND POLYCYTHAEMIA VERA (PV)
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Background. The chronic myeloproliferative disorders ET and PV are relatively common disorders with higher prevalence in the elderly. These disorders can lead to major morbidity and mortality through both disease progression/transformation and vascular events. A number of agents are available for cytoreduction in these disorders. Busulfan, a non-specific alkylating agent, has been in therapeutic use for over 50 years. Although other agents are often preferred (in part due to concerns over leukemogenesis), Busulfan usually means less clinic attendance which makes it particularly useful in the elderly. Aims: To analyse the effectiveness of Busulfan in terms of blood count control and the inci-

dence of thrombotic complications. To assess the incidence of side effects (mainly as leukaemia transformation and lung fibrosis).Methods: We retrospectively analysed the clinical records of 53 patients who received oral Busulfan for a diagnosis of ET (32) or PV(21) during the period Jan 2006 - Jan 2011. We assessed for vascular events, leukaemia transform-
ation, platelet count / Hct at 0 months, 6 months and 12 months from commencing Busulfan. We recorded the indication for utilising Busulfan as opposed to other cytoreductive agents. Results: 53 patients were analysed (mean age at commencing Busulfan 81 years, range 44-94). Mean cumulative Busulfan dose was 270.5mg (range 18mg - 1106mg), given as intermittent short courses. A total of 2,485 patient-months of Busulfan therapy (mean 45.9 months per patient) were analysed. There was only 1 leukaemia transformation (possibly related to Busulfan). There were 9 cases of lung fibrosis (of which 1 defined as definite arterial event (TIA) and 1 possible venous event (femoral DVT), thus there was only 1 clear (+1 possible) treatment failure in a cumulative 202 patient years of treatment. PV patients had a mean Hct at commen-
cement of Busulfan of 0.492 l/l. At 6 months post commencement of Busulfan, mean platelet count was 478x10^9/l, and at 12 months mean Hct was 0.454 l/l. For ET patients mean platelet count at commencement of Busulfan was 871x10^9/l. At 6 months post commencement of Busulfan, mean platelet count was 478x10^9/l, and at 12 months mean platelet count was 407x10^9/l. Of the 33 patients who received Busulfan as a first line cytoreductive agent, logistical ease was recorded as the reason in 32 patients. 28 patients received Busulfan as a second line cytoreductive agent; 5 patients switched to Busulfan due to mouth/skin ulcers with Hydroxyradamidine; 8 patients were switched to Busulfan for greater logistical ease. Summary/Conclusions: Oral Busulfan for both ET and PV is both safe (with a low rate of leukaemagenesis and pulmonary fibro-

1373 THE RISK PROFILE FOR THROMBOTIC EVENTS IN EARLY PREFIBROTIC PMF - LEUKOCYTOSIS IS A RISK FACTOR FOR ARTERIAL THROMBOSIS IN EARLY PREFIBROTIC PMF BUT NOT IN ET
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Background. There is strong evidence indicating that the clear-cut separ-
ation of prefibrotic primary myelofibrosis (pPMF) from essential myeloproliferative neoplasm ask for re-assessment. We aimed to eval-
uate whether patients with pPMF have a distinct risk profile for vascu-
tary events.Methods: Risk and risk profiles for vascular complications were determined in 87 patients from our database with a valid diagno-
sis of pPMF according to the WHO 2008 criteria and compared to a cohort of 127 patients diagnosed with WHO-defined ET. Results: Leuko-
cytosis and a JAK2V617F mutated genotype emerged as significant risk factors for arterial thrombosis after diagnosis in pPMF, whereas in WHO-
diagnosed ET generic vascular risk factors such as arterial hypertension and diabetes mellitus enhanced the risk for arterial thrombosis. Conclu-
sions: Our results challenge the current knowledge of established and suspected risk factors for thrombosis and certainly need to be confirmed in larger studies. If validated, the finding of a different relevance of cer-
tain risk factors within the increasing variety of sub-categories will cer-
tainly change the current treatment strategies and help to better allocate patients to the appropriate treatment.

1374 FIRST RESULTS FROM THE GREEK ESSENTIAL THROMBOCYTOSIS REGISTRY
D Sotropoulos, T Marinakis, M Tsirigotis, M Ilias, V Papadopoulos, E Papadakis, E Spanoudakis, I Kotsiadinis, A Poulis, A Vassou, A Pigaditou, A Balta, M Dimou, M Kotsopoulo, C Matsouka,16th Congress of the European Hematology Association

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had a significant haematologic response, with an increase in haemoglobin. A third patient treated with both lenalidomide and erythropoietin became transfusion independent a month after lenalidomide was commenced. It was possible to discontinue his erythropoietin.

Results

Methods: We describe 5 patients (aged 55-70 years) with an established diagnosis of myelofibrosis - five with idiopathic myelofibrosis and one with myelofibrosis secondary to Polycythemia vera (PV). Among ET incidence were 3% and 5% were females. They have been treated with antiagregant and anticoagulant therapy. Six have been treated with idroxisuarea. All of them are Jak2 V617F positive. Two patients had SVT before CMNs diagnosis, 2 of them splenectomy. The other 2 CMNs patients have had a SVT diagnosis during follow-up. 94% of SVT/CMNs patients had one pro-thrombotic risk factor, at least (factor V Leiden, Protein C deficiency, hyperhomocysteinemia) and 50% had 2 or more associated defects. The remaining CMNs patients had a less significant prevalence of pro-thrombotic risk factors and developed other venous thrombosis: wbc, platelets values and Jak2 V617F mutation correlate with the thrombotic event. The 7 patients had SVT before CMNs diagnosis no other major thrombotic event has been occurred during follow-up.

Conclusions: Even SVT has a low incidence in CMNs patients, we discuss on the potential benefit of searching for other prothrombotic risk factors in the whole population in order to properly treat with anticoagulant therapy.

1376

INCIDENCE AND RISK FACTORS OF SPLANCHNIC VENOUS THROMBOSIS (SVT) IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS (CMNS)

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Several data in literature underline the clinical and pathogenetic role of JAK2 V617F mutation in CMNs. Its role in additional thrombotic risk in CMNs patients is still under discussion. The same mutation has been identified as occult marker in several SVT patients; in particular, 45% of patients with Budd-Chiari syndrome (BCS) and 34% of portal vein thrombosis patients (PVT) have associated CMNs. The main BCS patients without CMNs present an additional congenital or acquired thrombosis risk factor. The aim of our study is to evaluate SVT incidence in CMNs patient followed up in our Institution and the presence in the same population of other prothrombotic risk factors. Out of the 405 CMNs Ph1 negative patients retrospectively evaluated (jun 2000-jun 2010), 9 females and 2 males, (2.2%) presented SVT. 6 of them had essential thrombocytemia (ET) 2 idiopathic myelofibrosis (IM) and 1 polycytetemia vera (PV). Among ET incidence were 3% and 5% were females. They have been treated with antiagregant and anticoagulant therapy. 6 have been treated with idroxisuarea. All of them are Jak2 V617F positive. 7 patients had SVT before CMNs diagnosis, 2 of them splenectomy. The other 2 CMNs patients have had a SVT diagnosis during follow-up. 94% of SVT/CMNs patients had one pro-thrombotic risk factor, at least (factor V Leiden, Protein C deficiency, hyperhomocysteinemia) and 50% had 2 or more associated defects. The remaining CMNs patients had a less significant prevalence of pro-thrombotic risk factors and developed other venous thrombosis:

1377

MORPHOMETRIC ANGIOGENESIS PARAMETERS FOR INDOLENT AND AGGRESSIVE NON-HODGKIN’S LYMPHOMA

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There is much evidence about importance of angiogenesis in development and progression of solid tumors. The role of angiogenesis, as an indicator of higher malignant potential in non-Hodgkin's lymphoma, is not clear at the moment. Morphometric characteristics of microvessels in lymph node sections in previously untreated patients with small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) and diffuse large B-cell lymphoma (DLBCL) were studied and relationship between angiogenesis and NHL histological malignancy grade was also evaluated. Lymph node biopsies samples of 30 newly diagnosed patients with SLL/CLL (n=10) and DLBCL (n=20) were studied. All samples were fixed in 10% buffered formalin solution and embedded in paraffin. Microvessels were visualized by immunohistochemical staining for anti-F8 antibody. In the area showing the most intense vascularization (i.e. the “hot spot”), microvessel density (MVD), total vascular area (TVA), as well as the size related parameters were estimated, by using image analysis program®analySIS®. Results. Number and size-related microvessels angiogenic morphometric parameters were statistically higher in group with DLBCL compared with SLL/CLL: MVD (p=0.002), TVA (p=0.0001), area (p<0.0001), perimeter (p=0.0001), minor axis length (p=0.0001) and major axis length (p<0.0001). It is to be noted that correlation existed between TVA and MVD in DLBCL and SLL/CLL. Conclusions. The present study supports the view that angiogenesis correlate with histological grade of NHL.

1378

NUCLEAR FACTOR-KB ACTIVATION IN PRIMARY LYMPHOMA OF BONE

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There is much evidence about importance of angiogenesis in development and progression of solid tumors. The role of angiogenesis, as an indicator of higher malignant potential in non-Hodgkin's lymphoma, is not clear at the moment. Morphometric characteristics of microvessels in lymph node sections in previously untreated patients with small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) and diffuse large B-cell lymphoma (DLBCL) were studied and relationship between angiogenesis and NHL histological malignancy grade was also evaluated. Lymph node biopsies samples of 30 newly diagnosed patients with SLL/CLL (n=10) and DLBCL (n=20) were studied. All samples were fixed in 10% buffered formalin solution and embedded in paraffin. Microvessels were visualized by immunohistochemical staining for anti-F8 antibody. In the area showing the most intense vascularization (i.e. the “hot spot”), microvessel density (MVD), total vascular area (TVA), as well as the size related parameters were estimated, by using image analysis program®analySIS®. Results. Number and size-related microvessels angiogenic morphometric parameters were statistically higher in group with DLBCL compared with SLL/CLL: MVD (p=0.002), TVA (p=0.0001), area (p<0.0001), perimeter (p=0.0001), minor axis length (p=0.0001) and major axis length (p<0.0001). It is to be noted that correlation existed between TVA and MVD in DLBCL and SLL/CLL. Conclusions. The present study supports the view that angiogenesis correlate with histological grade of NHL.

1378

NUCLEAR FACTOR-KB ACTIVATION IN PRIMARY LYMPHOMA OF BONE

F Heyning, I Koeni, A Szepesi, A Matolcsy, P Hogendoorn, P Jansen

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Background. Primary lymphoma of bone (PLB) is a rare type of extra-nodal non-Hodgkin lymphoma (NHL). This aggressive disease has a relatively indolent clinical behaviour and a favourable outcome. Previous reports have demonstrated a subdivision of PLB in germline (GC) and non-GC phenotype by immunohistochemistry. Recent scientific interest has focused on elucidating the role of nuclear factor (NF-κB) pathway in lymphomagenesis and its possible value as a therapeutic target. In nodal B-cell non Hodgkin lymphomas, constitutive activation of NF-κB appears to be especially involved in tumour cell survival in the non-GC type of DLBCL, although it is not exclusively restricted to this subtype. Aims. We here investigate NF-κB activation in PLB with GC and non-GC phenotype. Material and methods. In order to assess involvement of NF-κB activation in PLB, immunohistochemical staining procedures for NF-κB family members p50, p52 and p65 were performed on paraffin-embedded tumour tissues of 50 cases of PLB. Results: Nine cases (16% of our cohort) showed nuclear positivity for p50, and one case showed nuclear co-expression of p52. Positivity for p50 was not restricted to either GC- or non-GC phenotype of the tumour (20% and 25%, respectively), or related to an inferior prognosis or treatment resistance. p65 did not show significant nuclear staining. Summary and conclusions. The immunohistochemical nuclear staining for p50 in 16% of the cases suggests constitutive activation of NF-κB via the classical pathway in a minority of PLB patients. In contrast to other extranodal types of DLBCL, there was a lack of nuclear co-localization of p65 staining, which may suggest a deviation in the NF-κB pathway activation. The alternative pathway of NF-κB activation does not appear to be involved, as only one case showed significant nuclear staining for p52. Finally, nuclear expression of p50 was not preferentially detected in non-GC type or GC-type PLB, or related to an inferior prognosis.

1379 DIAGNOSIS OF NON HODGKIN’S LYMPHOMAS (NHL) BY FLOW CYTOMETRY (FC) ON LYMPH NODE CELL SUSPENSIONS AT BLIDA, ALGERIA

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Background. The diagnosis of malignant lymphoma is primarily based on histological examination. The FC is a powerful tool for cell analysis that helps a closer definition of cell proliferation. Aims. The aim of our study was to compare the FC analysis of cell suspensions obtained by grinding lymph node biopsy or fine-needle aspiration with histologic study. Methods. The diagnosis of NHL is confirmed by histological study on lymph node biopsy. The biopsy was divided into two fragments: one for pathologic studies, the other is crushed and put into a culture medium to obtain a cell suspension; If the biopsy is deferred, a cell suspension obtained, cells are washed with phosphate-buffered saline to remove cytophilic antibodies and then treated by FC. FC immunophenotyping was performed with antibodies targeting T and B lymphoid populations (CD2, CD4, CD8, CD5, CD7, CD1a, αβ TCR, γδ TCR, kappa and lambda light chains, CD19, CD20, CD22, CD10, CD43, CD79a, CD79b, CD23, CD25, CD11c, IgM, CD56, CD34; the acquisition was made using four-color multiparameter flow cytometry. Detection of immunoglobulin light chain- restriction was used for diagnosis of B NHL. Detection of multiple abnormalities in the same cells and restriction of TCRs were used for diagnosis of T NHL. Results. Cell suspensions from 67 patients were included in this study: they involve 19 women and 48 men with an average age = 41 years (15 -76). A fine-needle aspirate of lymph node is performed in 42 patients (68.7%) with a mean of 13752 cells / µl (800-71 500) and lymph node crush in 21 patients (31.3%), with a mean of 11055 cells/µl. The NHL type was diagnosed according to the World Health Organization (WHO) classification. Patients were categorized into the following groups: B-cell NHL (n = 52) and T-cell NHL (n = 15). In the group where the monoclonality was confirmed by FC, when histology was available (45 cases), the correlation between FC and histology was 90.8%; discordance was related to 4 cases (9.2%) with advantage for FC in 2 cases and histology in 2 cases. Summary. Because the WHO classification of NHL incorporate immunophenotypic criteria, FC helps to better characterize these entities. In our study, FC has been contributory to the diagnosis of NHL, specifying monoclonality and has shown the relevance of benign lymphoid hyperplasia, undifferentiated carcinomas and Hodgkin’s disease. The CMF is a reliable and quick method for the evaluation of NHL.

1380 HIGH KI 67 INDEX IS A POOR PROGNOSTIC FACTORS FOR DLBCL TREATED WITH CHOP WITH OR WITHOUT RITUXIMAB

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Background: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease and patients exhibit a wide range of outcomes. The addition of Rituximab to CHOP chemotherapy (R-CHOP) has led to a marked improvement in survival and has altered the significance of previously recognized prognostic markers. Aim: we performed a retrospective analysis of 111 patients with de novo DLBCL to assess impact of clinical variables and biologic features on Response Rate (RR), Overall Survival (OS) and Progression Free Survival (PFS). Methods: 58 patients were treated with R-CHOP (post-Rituximab era) and 53 with CHOP (pre Rituximab era). For each case the following clinical data were measured: patient’s age, performance status, B-symptoms; serum lactate dehydrogenase (LDH); serum β2 microglobulin (B2µg); bone marrow involvement; bulky disease; extranodal disease; clinical stage and International Prognostic Index (IPI). A maximal tumor burden > 10 cm was defined as bulky disease. Immunohistochemical analyses were performed: Bcl2 expression was scored positive when at least 50% of tumor cells expressed the protein; proliferative index assessed by ki-67 immunostaining was scored using a cutoff of >80%. Results: the variables predictive of RR in CHOP group were B symptoms (P=0.047), age > 60 years (P=0.045), clinical stage (P<0.032), bone marrow involvement (P=0.045), bulky disease (P=0.007), IPI risk group (P<0.001) and bcl2 expression (P=0.002); in the R-CHOP group they were bulky disease (P<0.002), bone marrow involvement (P<0.049), IPI risk group (P<0.011), and Ki67 expression >=80% (P<0.049). At multivariate analysis, in patients treated with CHOP the independent prognostic factors associated with PFS were age, bulky disease, IPI risk group and bcl2 expression; associated with OS were Performance Status, clinical stage, IPI risk group and bone marrow involvement. By contrast, among patients treated with R-CHOP, the variables proving to be independent prognostic factors were bulky disease on PFS, and Ki67 expression >=80% on OS and PFS. Conclusions: Our data show that a high proliferative index could represent possible predictive factor of poor prognosis, which would help to identify a high risk subgroup of newly diagnosed DLBCL. Further large-scale and prospective studies will be required to confirm these results.

1381 GSTP1 POLYMORPHISM AS A PREDICTOR OF CLINICAL OUTCOME OF THE THERAPY OF NON-HODGKIN’S LYMPHOMA IN ELDERLY PATIENTS

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Background. The worldwide incidence rate of non-Hodgkin lymphoma (NHL) increases on 2-3 % annually. Therapy of NHL was improved over the last two decades, but prognosis for a half of patients still remains poor. Glutathione S-transferase P1 (GSTP1) is a member of the GST enzyme superfamly involved in the metabolism and detoxification of xenobiotics and reactive oxygen species. The gene that encodes the enzyme superfamily involved in the metabolism and detoxification of xenobiotics and reactive oxygen species. The gene that encodes the GSTP1 Ile105Val polymorphism could represent possible predictive factor of poor prognosis, which would help to identify a high risk subgroup of newly diagnosed DLBCL. Further large-scale and prospective studies will be required to confirm these results.
patients enrolled in the study and radiotherapy by indications. The response to the therapy was scored according to International Working Group criteria (1999, 2007). Genomic DNA from peripheral blood of all individuals was analyzed for identification of GSTP1 genotypes using TaqMan Polymerase Chain Reaction (PCR) allelic discrimination assays. Results. The genotypes of the GSTP1 gene in both control and patient groups did not differ significantly from those predicted by the Hardy-Weinberg distribution. Observed Val allele frequency was 0.52, similar to previous reports on allele frequencies for healthy Caucasians. The frequency of GSTP1 homozygous wild genotype was higher in patients with advanced stages (III-IV) than in patients with localized stages (I-II) (60.5 % versus 29.4 %, P = 0.02). Overall response rate after the first line therapy was better in patients with increasing number of Val alleles (68.25 %) compared to 27.1 % in patients with homozygous wild genotype (P = 0.02). We observed a correlation between GSTP1 homozygous wild genotype and unfavorable prognosis for patients with advanced NHL: among patients with relapse 82 % were of Ile/Ile genotype. Moreover we did not noticed early relapses in patients with Val/Val and Ile/Val genotypes, while patients with Ile/Ile have shown early relapses in 26.1 % of cases (P = 0.05). We carry on investigations with larger cohorts of patients for further assuring of our preliminary results. Conclusions. The results suggest that the Ile105 Val polymorphism of the GSTP1 gene may predict clinical outcome of the therapy of elderly NHL patients. Hence the investigations of GSTP1 polymorphism are very promising, since it might provide a possible application of this genetic marker as an independent prognostic factor of NHL.

1382
THE IMMUNOPHENOTYPE OF MALIGNANT LYMPHOCYTES IN SPLENIC MARGINAL LYMPHOMA ASSOCIATED WITH HEPATITIS VIRAL INFECTION
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Background. Follicular lymphoma (FL) is an indolent B-cell non-Hodgkin’s lymphoma (NHL) that comprises about 20% of all NHLs in Europe and North America. Gene expression and immunohistochemical studies suggested that immune microenvironment of FL plays an important role in clinical behavior and progression of the disease: expression is increased in T-cells in T-cells and macrophages and FDCs associated with a favorable outcome, whereas genes which derived from macrophages and FDCs were associated with inferior outcome. In 40-70% of FL cases the bone marrow (BM) is also involved at diagnosis which has been considered as a poor prognostic factor. Although lymph node (LN) and BM compartments of FL are related to the same neoplastic clone, several morphologic, phenotypic and genotypic differences have been reported between the tumor cells within these two compartments. The cytological grade of the tumor is usually lower in the BM than in the LN; FL cells of the BM frequently lose the expression of BCL-6 and CD10 and the mutation pattern of IgH variable region genes of FL cells shows many differences between BM and LN. Aims: To investigate the role of the microenvironment in the bone marrow involvement of FL, we performed immunophenotypical analysis of the reactive cell populations in the lymph nodes and corresponding bone marrows of 35 patients with FL. The microenvironment patterns of the BM infiltrates were compared to the corresponding features of the LN in cases with BM involvement and the LN microenvironment was compared in FL cases with and without BM involvement. Methods. Automated image-segmentation-based localization and quantitation was performed in whole digital slides of immunostained tissue microarrays of formalin-fixed paraffin-embedded tissue biopsies. Results: We found significantly more CD68+ cytotoxic T-lymphocytes, FOXP3+ regulatory T-lymphocytes, CD103+ macrophages and less PD1+-follicular B-helper T-lymphocytes in the BM than in the matching LN samples. Furthermore, we observed significantly less infiltrating CD8+ T-cells and CD68+ macrophages in cases involving the bone marrow compared to those localized only to the lymph nodes. Conclusions. Our study showed that the lower grade and proliferation of the BM could be explained by the different composition of the microenvironment compared to the BM. On the other hand, different tumor cell growth in the LN and BM may generate different microenvironment. Our study also suggested that cytotoxic T-lymphocytes and macrophages play a relevant role in the prevention of tumor cell propagation and migration and their elevated number in LNs prevents BM infiltration of neoplastic cells in FL.

1384
THYROID LYMPHOMA CONSISTENTLY DIAGNOSED BY FLOW CYTOMETRY OF FINE NEEDLE ASPIRATION BIOPSY SAMPLE
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Diagnosis of primary thyroid lymphoma (PTL) is most commonly based on histopathological (HP) and immunohistochemical (IH) examination of surgical specimen from thyroidectomy and occasionally on flow cytometry (FCM) of cell suspensions. The routine method (HP/IH)
is able to capture a tissue structure but is based on invasive biopsy, is
time consuming and sensitive to the quality of the slides. In addition, a
range of antibodies used in IH are limited. Furthermore, post-surgical
complications may postpone initiation of the curative systemic treat-
ment, and a permanent hormonal substitution is a rule. FCM of a cellu-
lar suspension obtained by the fine needle aspiration biopsy (FNAB) of
thyroid gland is a safe, rapid, and cost-effective procedure. A broad range
of antibodies can be used to determine a simultaneous expression of
numerous antigens as well as clonality. Disadvantage of FCM may be
possible loss of diagnostic cells and missing tissue structure. The thyroid
polyclonal lymphoid infiltrate in Hashimoto thyroiditis (HT) represents
the substrate from which PTL may arise. Assess the usefulness of
FNAB/FCM in the diagnosis of PTL. We identified 11 cases of PTL in a
database of 3000 lymphoma patients. Diagnosed by FNAB/FCM be-
tween 2000 and 2010. PTL cases were retrospectively reviewed by
comparing conventional cytological smears and FCM. Clinical presen-
tation included estimation of performance status (PS), lymph nodes sta-
tus, LDH level a previous history of HT. Lymphoma mononuclear cells
and polyclonal lymphoid reactive infiltrate cells associated with HT
were evaluated by antibodies to the range of antigens: CD45, CD10,
and polyclonal lymphoid reactive infiltrate cells associated with HT
tus, LDH level a previous history of HT. Lymphoma mononuclear cells
and polyclonal lymphoid reactive infiltrate cells associated with HT
may be used to determine a simultaneous expression of
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possible loss of diagnostic cells and missing tissue structure. The thyroid
polyclonal lymphoid infiltrate in Hashimoto thyroiditis (HT) represents
the substrate from which PTL may arise. Assess the usefulness of
PLASMOBLASTIC LYMPHOMAS WITH SKIN INVOLVEMENT

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Background. Plasmablastic lymphoma (PBL) is a rare subtype of non-Hodgkin's lymphoma with a rather poor prognosis. The first cases were characterized as oral lesions of HIV+ patients, and later on more extranodal cases were also found but up to now very few cases with skin involvement/origin of HIV- patients were reported. PBL usually has a strong association with immunosuppression (81% of cases are HIV+) and EBV-infection. PBL consists of typically CD20-, LCA-, CD138+, VS38c+ and lambda or kappa monoclonal immunoblast like large cells with eccentrically located vesicular nuclei with one or sometimes more prominent nucleoli and with a high proliferation rate. There is a strong overlap between plasmablastic myeloma multiplex and plasmablastic lymphoma and the differential diagnosis might be very difficult. Markers which can help the differential diagnosis are p53, Mum1, Pax5, EBER ISH. Aims. Here we report and review seven cases (one female and six males) of plasmablastic tumors of HIV-negative patients with skin involvement. Methods. Average age was 65 years at the diagnosis of the disease. Three of the cases had a medical history of myeloma multiplex which later progressed to an aggressive plasmablastic tumor. Another two patients had immunosuppressive therapy because of renal transplantation. We performed a thorough immunohistological analysis with 15 markers (CD20, CD79a, Kappa, Lambda, CD38, CD45, CD138, VS38c, Ki67, CD10, CD30, MUM, MUM1, Pax5, EMA, Pax5 and MUM). The most important differential diagnostic problem was to separate PBL from plasmablastic myeloma multiplex, which distinction can be made upon the exact medical history, clinical correlations and bone marrow investigation/staging supported by a coexpression of several immunohistochemical markers, but in doubtful cases only the EBV-detection could help us. Conclusions. Although skin involvement of the very aggressive plasmablastic lymphoma is an exceptionally rare occurring phenomenon it can occur not only in HIV+ patients but also without HIV-infection and therefore can cause very serious differential diagnostic problems. Morphologic and (immuno)phenotypic characterization might be insufficient for distinguishing plasmablastic myeloma multiplex and plasmablastic lymphoma. In these cases exact clinical data/correlations and EBV detection can help the most effectively.

TUMOR INFILTRATING CD 68 POSITIVE MACROPHAGES PREDICT SURVIVAL IN MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma (MCL) is a malignant lymphoma associated with a relatively aggressive clinical course and a median overall survival time of 3-4 years. Only limited data about tumor associated macrophages and their influence on survival in MCL exists. Methods. We analyzed the amount of CD68 macrophages in relation to the clinical outcome in patients with MCL. Lymph node biopsies of 77 untreated patients (17 women and 60 men) enrolled in two multicenter trials (1975-1985) with a median age of 66 years (range 41-86 years) were included in this study. Biopsy specimens were investigated immunohistochemically with monoclonal antibodies against CD68 (Ki-M1P). 10 High power fields (HPF) were evaluated by random. Results: Patients with low account (less than 10/HPF) of CD 68 positive macrophages had a median overall survival time of 38.2 months, compared to 24.2 months for patients with high macrophage content (more than 10/HPF). CD 68 positive macrophages. The Kaplan-Meier analysis showed a significant difference in the overall survival time (p=0.0027). Conclusions. Patients with mantle cell lymphoma and a low number of CD 68 positive macrophages have a better prognosis and can predict outcome.

ACCOUNT OF CD 8 AND FOXP 3 POSITIVE T CELLS PREDICT SURVIVAL IN MANTLE CELL LYMPHOMA

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Background. The role of tumor infiltrating T-Cells in malignant B-Cell lymphomas is discussed controversial. There are only limited data on CD 8 and FOXP3 positive cells in mantle cell lymphoma. Methods: 81 biopsy specimens of patients (64 men and 17 women) with mantle cell lymphoma and a median age of 64 years (range: 41 to 86 years) were included in this study. The slides were stained immunohistochemically with CD3, CD8 and FOXP3. Positive T-cells of 10 High power fields (HPF) were counted and the average value was calculated.

APOTOPSIS REGULATING PROTEINS AND THE LEVEL OF APOPTOSIS CAN PREDICT SURVIVAL IN MANTLE CELL LYMPHOMA

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Background. The deregulation of apoptosis is has been implicated in cancer, autoimmunity and degenerative disorders. At the molecular level an extrinsic death receptor pathway and the internal intrin-
sic (mitochondrial) pathway have been described. Only limited data exist on the expression of proteins involved in apoptotic pathways in mantle cell lymphoma are limited. Methods We investigated the expression of p53, the indicator of DNA damage, and of proteins involved in the regulation of the internal intrinsic mitochondrial pathway (BCL2, Bax) and of effector proteins of apoptosis (caspase 8, caspase 3) in 93 cases of MCL mantle cell lymphoma and correlated the expression with the clinical outcome. Results Similar to previous studies, we found that p53 expression was associated with a shorter overall survival. In contrast to diffuse large B-cell lymphomas, cases expressing the anti-apoptotic protein BCL2 had a favourable outcome. Interestingly, high levels of apoptosis in the tumor before treatment, as indicated by expression of active caspase 8, is are a strong indicator of poor clinical outcome (p<0.001).

Conclusions These data indicate, that the level of apoptosis itself is a strong prognostic marker in mantle cell lymphomas.

1391
DIET AND NON-HODGKIN’S LYMPHOMA RISK
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Background The role of dietary factors in the epidemiology of Non-Hodgkin’s lymphoma (NHL) remains largely undefined. Dietary habits may play a role in the etiology of NHL by influencing on immune system. Design and methods We analyzed dietary patterns and the risk of NHL in the case control study, which includes 170 NHL cases (mean age 52 years) and 190 controls (mean ages 46 years). All subjects completed a validated food-frequency questionnaire. Dietary pattern investigated in nine groups and separately. Crosstab tables were used to estimate the odds ratios (OR) and the corresponding 95% confidence intervals (CI) and P trend. Results Consumption of highest versus lowest quartile of proteins (OR, 8.088 Ptrend=0.000), fats (OR, 6.17 Ptrend=0.000) and sweets (OR, 8.806 Ptrend=0.000) were associated with a significantly increased NHL risk. Inverse association was found for fresh fruits (OR, 0.117 Ptrend=0.000) and vegetables (OR, 0.51 Ptrend=0.010). Conclusions An association between dietary intake and risk of NHL is biologically plausible because of immunosuppressive effect of fat and animal proteins and antioxidant properties of vegetable and fruits. It is recommended to encourage the general population to increase dietary fiber and to limit fat, red meat and sweet consumption.

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CHEMOTHERAPY WITH ALTERNATING REGIMEN MICMA/IGEV IN ELDERLY PATIENTS WITH REFRACTORY DLBCL: A FIGHT AGAINST WINDMILLS?
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Background In Europe, more than 50% of the lymphomas arise in elderly patients older than 65 years. Now R-CHOP regimen is the gold standard in treatment of aggressive lymphomas in this patient subset. Salvage strategies are needed for these patients who are not cured with first line therapy, since high dose chemotherapy frequently is not suitable. 2nd line therapy with DHAP produces scarce results. Aims To evaluate safety, feasibility, efficacy of 2nd line treatment in patients older than 60 yo with combined chemotherapy with MICMA - IGEV Methods. In two years we treated 20 patients with resistant diffuse large B cell lymphoma (DLBCL) with MICMA-IGEV alternating chemotherapy cycles (MICMA: methylprednisolone 500mg/mq gg1-5, mitoxantrone 10 mg/mq gg1, cytarabine 2 g/mq gg2, carboplatinum 100mg/mq gg1-4; IGEV: hyphosphamide 2 g/mq gg1-4, gemcytamine 800 mg/mq gg1 and 4). Chemotherapy cycles were administered every 21 days if only complete hematopoietic recovery was occurred. M/F was 12/8, median age was 72.5 years (R65-84). Results 15 patients (75%) received 4 chemotherapy cycles, 1 patient (5%) received 6 cycles, 4 patients (20%) received 2 cycles. Median cycles administered were 4 (R2-6). At 29 days all patients were alive. 3 patients (15%) had kidney toxicity. Only 2 patients died for therapy-related toxicity. 5 patients required 25-33% chemotherapy dose reduction for organ toxicity. 8 patients (40%) delayed chemotherapy administration for G3-G4 neutropenia or thrombocytopenia. Summary/Conclusion In literature patients of all age resistant/relapsed after 1st line chemotherapy and treated with 3 ESHAP or 2 DHAP cycles had a 5 years survival of 25%. Elderly patients with more of 60 years with relapsed/resistant DLBCL and treated with 2-4 cycles of DHAP showed a median survival of 9 months. Our patients treated with 4 MICMA/IGEV alternating cycles showed a median survival of 12 months (R1-29 months). 4 cycles of alternating MICMA/IGEV chemotherapy seems to be relatively safe, feasible and effective in patients with more of 65 yo, also with comorbidities. These data need of confirmation on a large cohort of patients.

1393
HELCIBATER PYLORI ERADICATION WITH SEQUENTIAL THERAPY IN GASTRIC B-CELL, LOW GRADE, MALT-LYMPHOMA PATIENTS: PRELIMINARY RESULTS
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Background H. pylori eradication is recognized as first-line therapy in early stage, B-cell, low-grade, gastric MALT-lymphoma patients. However, cure rate following standard triple therapies for such an infection is decreasing worldwide. This study assessed the efficacy of a 10-day sequential for H. pylori eradication in these patients. Methods All patients diagnosed with gastric lymphoma were considered. Patients underwent upper endoscopy with biopsies, echo-endoscopy of gastric mucosa, total-body CT, and bone marrow biopsy: Only those patients with H. pylori-associated, low-grade MALT-lymphoma in stage I-II were enrolled. Patients received a sequential therapy including omeprazole 20 mg plus amoxicillin 1 g for the first 5 days followed by omeprazole 20 mg, clarithromycin 500 mg and tindazole 500 mg for the remaining 5 days, all given twice daily. Both bacterial eradication and lymphoma remission was considered achieved when 2 consecutive histological examinations were negative Results. Data of the first 10 patients (5 M, 5 F, median age 65 years, R51-77 years, stage I: 5; II: 5) were analyzed. Median follow-up was 12 months (range 8-22 months). H. pylori eradication was achieved in all 10 (100%) patients, whilst lymphoma remission occurred in 8 (80%) cases. One patient achieved lymphoma remission with radiotherapy following anti-CD20 monoclonal therapy failure, whilst the other patient is still in follow up (10 months following bacterial eradication). Conclusions H. pylori eradication rate following standard triple therapy is decreasing. This is the first study showing a high efficacy of the 10-day sequential therapy for curing such an infection in MALT-lymphoma patients.
patients with unknown risk could induce the onset. In the period 2008-2010, we analyzed prospectively 40 patients with newly diagnosed non-Hodgkin lymphoma by a serial samples of pro-BNP and Troponin I, before each cycle of chemotherapy inclusive anthracycline, at day +1 by the execution, at the end of therapy and during the follow up at 3 and 12 months. Before therapy and at the end was assessed left ventricular ejection fraction by echocardiography. On the basis of risk factors have been identified two groups (see Table I). Patient's age or history of heart disease was classified as high risk and received the CHOP scheme with the replacement of doxorubicin with MYOCET (non-pegylated liposomal doxorubicin). While the second group performed the standard therapy with CHOP scheme. Both groups received a total of 6 cycles every 3 weeks. Results. 52 out of 40 patients achieved a complete remission, 7 patients a partial remission and only one has progressed. At 1 year, 31 patients are alive and well. The PRO-BNP increased during the cycles of therapy in 85 to 40 (see Table II) and troponin I is increased in 3 patients (all in the group treated with conventional CHOP). At 3 months and 1 year 2 out of 40 patients have pro-BNP increased while no one with increased troponin I. At 1 year after the treatment in the group treated with CHOP-MYOCETTM 7 patients showed an impairment (not life threatening) of cardiac disease, while in the group treated with conventional anthracyclines three patients developed a new severe heart disease. Conclusions. The PRO-BNP in our study did not show a prediction as it increases in almost all patients. Appeared the most promising Troponin I, although given the small sample size will require more study. The use of the anthracycline liposomal MYOCETTM confirmed its efficacy and tolerance without inducing new heart disease in a group of patients at high risk for heart disease and ancient age.

Table I

<table>
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<th>N°</th>
<th>CHOP-MYOCET™</th>
<th>CHOP</th>
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<tr>
<td>25/40</td>
<td>15/40</td>
<td>9/6</td>
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<tr>
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<td>9/6</td>
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Table II.

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</tr>
<tr>
<td>PP 0/25 1/15</td>
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<tr>
<td>INCREASE OF PRO-BNP DURING CYCLES</td>
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<td>INCREASE OF TROPONIN I IN CYCLES</td>
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<td>INCREASE PRO-BNP TO 3 MONTH</td>
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<tr>
<td>LIVE AT 1 YEAR</td>
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<tr>
<td>DEVELOPMENT OF HEART AT 1 YEAR IN ALL GRADES</td>
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<td>INCOMING OR SEVERE HEART DISEASE</td>
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STUDY REGARDING THE ASSOCIATION BETWEEN MULTIPLE PRIMARY CANCERS THAT INCLUDES A MATURE B-CELL NEOPLASM AND THE METABOLIC SYNDROME

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Background. It is known that cancer patients have a 20% higher risk of new primary cancer compared with the general population. We have published studies showing that patients with non-Hodgkin lymphoma have more frequently components of metabolic syndrome compared to subjects without cancer. Aim: We aimed to study the presence of metabolic syndrome components in patients with multiple primary cancers that include a mature B-cell neoplasm comparing with those with only a mature B-cell neoplasm. Methods. Of the 665 patients with hematological malignancies who were registered in the Department of Hematology of Hospital Emergency Sibiu, during January 2006 - January 2011, we had selected 424 people with mature B-cell neoplasms, and among them we had chosen all the 21 multiple primary cancers (group A), which we compared with a group of 62 consecutive patients with mature B-cell neoplasms which were in our evidence in January 2011 (group B). We made a comparative analysis of the components of metabolic syndrome and hypercholesterolemia in the two groups. The results were statistically analyzed. Results. The average age of patients in group A was 66.57 +/- 12.32 years, and in group B - 63.57 +/- 9.98 years. The average time to onset of the 2nd cancer was 3.52 +/- 6.24 years.

Among patients with mature B-cell neoplasms, 4.95% had multiple primary cancers: 6 - skin carcinoma, 2 - lung cancer, 2 - malignant melanoma and one - gastric cancer, colon, rectum, kidney, breast, ovarian, metastatic with unspecified starting point, acute myeloid leukemia, acute promyelocytic leukemia, myelodysplastic syndrome, chronic myeloid leukemia and polycythemia vera. One patient had triple neoplasia. Patients in group B were more frequently obese (71.43% vs. 57.14%) and had hypertriglyceridemia (40.52% vs. 38.10%), while those in group A had more frequently high blood pressure (57.16% vs. 41.94%) and diabetes mellitus (53.35% vs. 20.97%). The average of metabolic syndrome components was higher in group A patients compared with those of B (1.66 +/- 1.24 to 1.61 +/- 1.11). The presence of two cancers predisposes to accelerate catabolic processes, which may explain the reduction in obesity and hypertriglyceridemia. In this group, which is more exposed to stress and various therapies, including corticosteroids, are more frequent high blood pressure and diabetes mellitus, and in group B - 63.57 +/- 9.98 years. The differences were not statistically significant. Only ischemic cardiopathy is more common in group A patients (p=0.025). Conclusions. A significant proportion of patients with mature B-cell neoplasia still has one or two neoplasms. While patients who have only mature B-cell neoplasia frequently suffer from obesity, hypertriglyceridemia, others with multiple primary cancers, including mature B-cell neoplasms, have high blood pressure, diabetes and more components of metabolic syndrome more frequently compared to the first. Studies on large groups of patients are needed so that results can have statistical significance.
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T CELL/HISTIOCYTE RICH LARGE B-CELL LYMPHOMA (TRHLCL): CLINICAL CHARACTERISTICS, PROGNOSIS AND MANAGEMENT: A REPORT ON 17 PATIENTS

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Background. T cell/histiocyte rich large B-cell lymphoma (TRHLCL) is a rare subtype of diffuse B cell non Hodgkin lymphomas. Because of a considerable similarity to nodular lymphocyte predominating Hodgkin’s lymphoma (NLPHL) and classic Hodgkin’s lymphoma (cHL), an accurate diagnosis is based on exhaustive immunohistochemical and molecular genetic analyses. Aims. To analyze the laboratory and histology in patients (pts) with TRHLCL with selected aspects of the course and survival in relation to different choices of therapy. Patients and Methods. Seventeen pts were studied (m/f = 11/6, median age 41 years, range 28-72). Comprehensive immunocytochemistry and imaging methods were used to reach the diagnosis and stage of TRHLCL. The B symptoms were present in 94% of pts, peripheral lymphadenopathy in 82%, splenomegaly in 70%, and hepatomegaly in 92%. Mediastinal and abdominal lymphadenopathy were evidenced in 65% and 82% of pts, respectively. Most pts (65%) had a nodular type of disease. Primary extranodal disease was diagnosed in 23.5% of pts. Increased serum levels of LDH and β2-microglobulin were used as single proliferative measures of disease, thrombocytopenia, and leukocytopenia were present in 53.8%, 53%, and 18% of pts, respectively. Most pts were in a IVB stage of disease with a high average IPI score. The R-CHOP protocol was applied in eleven pts. Six patients were treated with CHOP, COD, ESHAP, R-EPOCH, ProMACe CytA/BOM and DHAP protocols each. Complete remission was achieved in 11.8% of pts and partial remission in 61.5%. In 25.5% of pts disease progressed despite the chemotherapy. In the collective median survival was 36 months. Conclusions. The TRHLCL is an aggressive lymphoma and the comparison of pts with this disease and those with diffuse large B cell lymphoma reveals no significant difference in survival.

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PROGNOSTIC FACTORS IN THE TREATMENT OF PRIMARY EXTRANODAL DIFFUSE LARGE B-CELL LYMPHOMA WITH RITUXIMAB BASED IMMUNOCHEMOTHERAPY

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Background. Primary extranodal lymphoma (ENL) constitute 25-50% of all non-Hodgkin lymphomas. About half of extranodal lymphoma are diffuse large-B-cell lymphoma subtype. The most common extranodal DLBCL localizations are stomach, central nervous system (CNS) and testis. Aims: The aim of this study was to determine the clinical and laboratory differences between patients with nodal (NL) and ENL DLBCL at the time of diagnosis, and the effect of clinical and laboratory parameters on overall and progression free survival (OS, PFS) in patients with the extranodal lymphomas. Material and methods: The study included 95 patients with CD20 positive diffuse large-B-cell lymphoma treated at the clinic in the period 2003 and 2009, 08 new cases of nasal NK/T-cell lymphoma were diagnosed at the clinic. The aim of this study was to investigate the frequency and clinicopathologic features of nasopharyngeal extra nodal NK/T-cell lymphoma, and evaluate the outcome of this disease. Patients and methods: Between 2003 and 2009, 08 new cases of nasal NK/T-cell lymphoma were diagnosed in our institution. The diagnosis is essentially based on the clinical presentation of extra nodal ulcerative lesions in the upper aerodigestive tract and histopathologic analysis of biopsies using immunohistochemistry. Results. The median age was 46 years (ranges 19-77); male/female ratio was 4/4. The clinical features are: pain, obstruction, foul smell, discharge, and bleeding. Primary nasal lesions were ulcerative and are locally invasive and necrotic. This aggressive lymphoma remains localized in the nasal primary site in most cases. Systemic dissemination (stage IV) occurs in one patient (skin, testis) The histological findings showed angiocentric, necrosis, and pleomorphic infiltration. The immunophenotypes were: CD3+, CD56+, and CD45+ in all patients. The Ann Arbor stage was IE, IIE in 7 cases, and IV in one patient. The patients were classified as having low IPI scores, and only two of them had bulky disease. Treatment modalities were given as follows: chemotherapy alone for 5 pts, chemotherapy with involved radiation therapy for 2 with a median dosage of 40 Gy. The chemotherapy regimens included CHOP (Cyclophosphamide, Doxorubicin, Vincristin, and Prednisone) for 5 pts on first line, but they were relapsed and received an L-Asparaginase based salvage regimen (L-asparaginase, velbe, and dexamethasone). Five patients (62%) were responsive to the treatment. Three patients achieved complete response; two of them obtained partial remission. The overall response rate (CR+PR) was 62, 5%. The 3-year overall survival rate was 57%. Conclusion. The nasal, nasal-type T/NK-cell lymphoma is a rare and distinct clinico-pathological entity. The Results of this clinical study indicated that the L-asparaginase-based regimen significantly improved the response rate. But the overall outcome in nasal NK/T cell lymphoma is poor.

1400

DETECTION OF RELAPSE IN PATIENTS POST TREATMENT WITH DIFFUSE LARGE B CELL LYMPHOMA.- THE ROLE OF BLOOD TESTS

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Introduction. Blood testing in routine outpatient follow up of patients who have had successful treatment for Diffuse Large B Cell Lymphoma (DLBCL) is not helpful to predict or confirm relapse. There has been no conclusive evidence suggesting that blood tests help with predicting or confirming relapse post DLBCL treatment. However, how and when to perform routine practice in clinics. Aims. This study was done to provide evidence on whether blood tests are useful in the outpatient follow up clinics. Methods. A Retrospective study was undertaken to investigate all
patients with a diagnosis of DLBCL, treated at The Haematology Department in Plymouth University Hospital, Derriford, from 2005 to 2009. We selected patients with DLBCL who completed their chemotherapy and were in good partial remission or complete remission. Information was obtained from medical notes, X-ray PACS system and hospital laboratory results system. We looked at the all the full blood counts and clinical outcomes in the outpatients clinics. Results. Total number of patients: 62 patients. The median number of days from diagnosis to CT was 87 yrs. 38/62 (61%) had lymph phadenopathy on presentation, 5/62 (8%) had thyroid involvement, 6/62 (13%) had GI symptoms, 6/62 (8%) had bone involvement and 6/62 (9%) had other involvement which included skin and parotid node. 5/62 (8%) of the patients in the study showed partial response after chemotherapy, 14/62 (22%) showed complete remission or very good partial response after standard chemotherapy. Median follow up period was 25 months (range 3 months-60 months) Median number of OPD clinic per patient was 6 (range 1-22 clinics) in the study period. Median number of OPD blood tests done per patient in the study period was 6 (range 1-22 blood tests). 76/400 (19%) of the full blood count were abnormal. Of the abnormal blood tests - 74/400 (19%) of the FBC showed chronic anaemia, 1/47 (1%) showed thrombocytopenia and 1/47 (1%) showed CLL. 8/62 (13%) of the patients relapsed in the study period. None of the patient who relapsed had any change in their blood counts to indicate or aid to diagnose relapse. All patients who relapsed presented with symptoms of lymphoma - lymphadenopathy, 6/62 (10%), dyspnoea, stridor and 1/8 (12%) had CNS symptoms at relapse. Conclusions. This observational study shows that blood tests do not help in predicting or diagnosing relapse in patients following successful therapy. Majority of the abnormality seen on blood counts were due to other unrelated medical conditions. All relapses were diagnosed in patients presenting with a specific symptoms and/or signs. Following this study, it was noted that, patients found reassurance if their blood counts were normal, however once patients were educated on the blood tests being uninformative with regards to their follow up, their safety blanket association with blood tests was significantly reduced. Therefore, in an Out - Patient Department, rationalisation of blood tests can be done economically and without having an adverse outcome on patient care. In the current economical climate this could produce beneficial savings for the department without compromising patient care.

1401
IMPROVEMENT OF PRURITUS IN SEZARY SYNDROME: LITERATURE REVIEW AND CASE REPORT

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solidation chemotherapy as initially planned, without consolidative radiotherapy. In a planned follow-up of 14 months (range 1–40), all patients are alive without disease progression. One patient presented respiratory failure due to pleural effusion after surgery, and had to undergo non-invasive ventilation for 4 days. Summary/Conclusions. In patients with DLBCL of the mediastinum, surgical restaging of 18F-FDG-avid residual mediastinal masses after initial chemotherapy, although not recommended large studies that tumor necrosis remains metabolically active. Restaging PET should be interpreted with caution to make treatment decisions, and the results of large prospective trials evaluating PET-guided treatment in DLBCL are eagerly waited.

**1403** BENDAMUSTINE AND RITUXIMAB IN RELAPSED OR REFRACTORY LOW-GRADE LYMPHOMAS

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Background: Bendamustine is a hybrid of a purine analogue and an alkylating agent, with activity against low-grade lymphomas in combination with rituximab (BR) and a good toxicity profile. Bendamustine has recently become available in clinical practice and experience is limited.

Aims: Herein we present our experience on treating relapsed or refractory low-grade lymphomas and mantle cell lymphomas with the combination of bendamustine and rituximab (BR). Patients and methods: Retrospective and observational study of all consecutive patients with relapsed or refractory low-grade lymphoma or mantle cell lymphoma treated with BR since April 24, 2008 to January 31, 2011. Modified Cheson criteria (2007) were used to assess response. Adverse effects were classified using the WHO toxicity criteria. Bendamustine (90mg/m² daily) was administered the first and second days of each cycle. Rituximab (375mg/m²) was administered a week before the first cycle, the first day of every cycle and four weeks after the last one. Cycles were administered every four weeks to a maximum of six. Patients were evaluable for response if they have received at least two cycles of BR. Statistical methods: Descriptive statistics and Kaplan-Meier survival analysis. Results: Thirteen patients (6 women and 7 men) were included. Mean age was 67.3 years and median age 70 years, range 45 to 82. Diagnosis was follicular lymphoma (FL) in six patients, mantle cell lymphoma (MCL) in three, small cell lymphocytic lymphoma (SCLL) in three and MALT lymphoma of the oral mucosa (relapsed in skin and conjunctiva) in one. Mean time from diagnosis was 5.4 years (2.6-10.5). Mean previous therapies was three (1-5); and two patients (one FL and one MCL) had undergone an autologous bone marrow transplantation. Seven patients received six cycles of BR, four patients 4 cycles and one patient 3 cycles. One patient received only two cycles, and the treatment was early terminated because of hepatitis C reactivation. Complete response (CR) was achieved in ten patients (76%) and partial response in one (7.5%). Two patients (15%) showed no response. Two patients with CR have relapsed (a SCLL and a MCL) at 19 and 21 months respectively, while the remaining eight maintain a CR. The median time to treatment failure was 19 months and the median survival was 21 months. The 12-month observation of response rate was 95.8%. Adverse events were grade 3 or grade 4 neutropenia in seven patients (53%) and grade 3 thrombocytopenia in two (15%). An episode of febrile neutropenia and another of coagulase negative staphylococcus bacteremia associated with indwelling catheter were reported. There was a transient hepatitis C reactivation with remission after BR termination. Five patients reported nausea and vomiting and two asthenia, both in grade 1 or 2. Conclusions. Treatment with BR was effective in a high percentage of our patients with relapsed or refractory low-grade lymphoma. Of note, tolerability and safety were good in elderly patients and responses were durable. More experience is needed to confirm the good profile of the combination in the long term.

**1404** FEASIBILITY OF HIGH-DOSE METHOTREXATE, TEMOZOLOMIDE AND INTRATHecal LIPOSOMal CYTARABINE (HD-MTX-TMZ-IT LC) FOR THE TREATMENT OF CENTRAL NERVOUS SYSTEM (CNS) LYMPHOMA

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Background. CNS lymphoma is an aggressive tumor. Combined systemic chemotherapy-radiotherapy may be associated with acute/delayed neurotoxicity. HD-MTX-TMZ appears to be an effective and relatively safe regimen. Adding IT LC may further improve therapeutic efficacy. Aims. We report our preliminary experience with HD-MTX-TMZ-IT LC used upfront or as salvage in 4 CNS lymphoma patients. Methods: Induction: MTX 3g/m² IV d 1, 10, 20, TMZ 100mg/m² d 1-5, maintenance (aSD pts): MTX 3g/m² d 1, TMZ 100mg/m² d 1-5, every month, for up to 5 cycles as long as response was documented. Fifty mg IT LC was given concomitantly, at least 14 days apart and at least 7 days from HD-MTX, up to 6 doses. Results: Pt 1, 56 y, male, primary CNS lymphoma, peripheral T cell lymphoma not otherwise specified, IT LC in one (7.5%). Two patients (15%) showed no response. Two patients (7%) had early terminated because of hepatitis C reactivation. Complete remission was early terminated because of hepatitis C reactivation. Complete remission was obtained very good PR after induction. She currently complains of residual perineal heaviness and mild difficulty waking, and is able to perform controlled micturition and defecation. Pt 4, 71 y, female, DLBCL, left cerebellar hemisphere, KPS 50%. HD-MTX-TMZ and concomitant IT LC were initiated as first-line therapy. She experienced G2 atrial fibrillation, resolved after inotropic treatment and never reappeared. No tumor maintenance was completed. However, after the 5th IT LC injection the patient presented with leg pain and difficulty walking, saddle hypoesthesia, urinary and fecal retention, suggesting couina-CAuda equina syndrome. IT therapy was thus withdrawn. Neurologic symptoms subsequently improved with physiotherapy and pregabalin, and stool softeners. She received 14 cycles of good PR after induction and sustained thus far. Conclusions. HD-MTX-TMZ-IT LC therapy appeared feasible and effective, even in elderly pts. CR/CRu was attained in 3/4 pts, with a response duration of 19+, 7, 11+ and 6+ months in pts 1, 2, 3 and 4, respectively. IT LC is associated with a number of neurologic complications, including Corus-Cauda equina syndrome. It appears to be in part related to the lumbar puncture procedure itself and not a LC-specific side effect. No delayed neurocognitive impairment has been observed so far.
were estimated in Lugano stage I, II, II, IIE and IV (p=0.079 and p=0.018). Low, low-intermediate, high-intermediate and high risk (p=0.065 and p=0.019), LDH < 450 IU/L and ≥ 450 IU/L (p=0.027 and 0.140), and surgery/CT and CT alone, (p=0.311 and p=0.301), respectively. In multivariate analysis, there was no independent predictive factors for survival. Conclusions. Atients treated with surgery followed R-CHOP were estimated to have higher survival rate than R-CHOP alone although there was no significant differences for survival rate. There was no significant prognostic factors for survival, but Lugano stage, IPI risk, LDH, and treatment modality could be possible prognostic factors for event free survival.

1406 POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN LIVER AND KIDNEY RECIPIENTS: A SINGLE INSTITUTION EXPERIENCE
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Background. Post-transplant lymphoproliferative disorders (PTLD) represent a heterogeneous group of disease ranging from reactive polyclonal hyperplasia to aggressive non-Hodgkin lymphomas (NHL) and in the majority of cases they are associated with Epstein-Barr virus (EBV). PTLD develop as a consequence of the severe impairment of T cell function and the related reduction of T cell control on EBV latently infected B cell due to immunosuppressive therapy after solid organ (SOT) or hematopoietic stem cell transplantation (HSCT). Aims. We analyzed the clinical features, treatment and outcome of a series of adult patients who developed NHL after liver and kidney transplantation. Methods. We retrospectively studied the clinical data of 16 patients that developed NHL occurred at our institution between 1998 and 2010. Results. 16 patients (M/F ratio 2:2) with a median age of 42 years (range 20-59) developed NHL after liver, 3 cases, and kidney, 13 cases, respectively. Previous liver diseases were: hepatitis C virus cirrhosis in two cases and primary sclerosing cholangitis in one case; previous kidney diseases were: glomerulonephritis in 10 cases and congenital syndrome in three cases. Median time from transplantation to PTLD was 111.5 (range 3-560) months. Six patients experienced acute transplant rejection that required intensification of immunosuppressive therapy. Hematologic diagnosis included 12 monomorphic PTLD (10 diffuse large B lymphoma, 1 peripheral T cell lymphoma, 1 anaplastic large cell lymphoma), 2 classical Hodgkin lymphoma-type PTLD, 1 lymphoplasma-cytic lymphoma and 1 extranodal marginal zone lymphoma. Histological diagnosis was made on lymph node and on extranodal tissue in seven and nine cases, respectively. Available histologic analysis of tumor tissue demonstrated that EBV was positive in 62% of cases. CD20 expression was available in all but two patients and was positive in 71% of cases. At diagnosis seven patients were in stage IV, two in stage III, one in stage II and 6 in stage I; median IPI and Mayo prognostic score were 2 and 1, respectively. Ten patients presented with extranodal disease; central nervous system (CNS) involvement was not observed. Eleven patients received immuno-chemotherapy (including anthracyclines) with rituximab and two only chemotherapy, among them only four patients required reduction of doses because of liver/toxicity, and one patient experienced infective toxicity (WHO grade 1-2). One patient was treated with only radiotherapy and two patients were observed because of indolent disease. After a median follow up of 51 months (range 1-128) from the end of therapy, 15 patients are alive and in complete remission; 7 patients died, 2 because of progressive disease and one patient died for progressive disease. Conclusions. Although the limited number of patients involved, and the peculiar subset of renal and liver allograft in which the risk of PTLD is low-intermediate, our study demonstrated that the majority of our patients developed aggressive NHL and an immuno-chemotherapeutic regime is feasible, well tolerated and allow to obtain an elevated percentage of remission.

1407 CLINICO-EVOLUTIVE ASPECTS AT 73 PATIENTS DIAGNOSED WITH HAIRY CELL LEUKEMIA ADMITTED IN FUNDENI CLINIC OF HEMATOLOGY BETWEEN 2000-2008
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Background. Hairy cell leukemia (HCL) is an indolent B cell lymphoproliferative disorder that affect middle aged males; the main symptoms at diagnosis are: fatigue, splenomegaly, pancytopenia, hemorrhagic syndrome, infections. The morphological marker of the disease in hairy cell, an activated memory B cell with characteristic immunophenotype (panB, CD5, CD23; CD25, CD11c, CD103; CD123). The new methods of diagnosis (flowcytometry, immunohistochemistry) allow the differential diagnosis between classical and/or another lymphomas (e.g. Splenic marginal zone lymphoma, with villous cells). The response to the treatment with interferon and/or purine analogues is very good, with long term remissions. Aims. To compare data obtained with data from other studies. Material and method: clinical and epidemiological retrospective study of 73 patients diagnosed in Fundeni clinic of Hematology between 2000-2008. Results : 62 patients were diagnosed with classical form, 11 with variant form of HCL, most of the patients were males, with a medium age of 57,1 years; the main features at diagnosis were: splenomegaly (>12 cm diameter at abdominal ultrasound)-55 cases; infections - 24 cases), cytopenias (mono, bi or pancytopenia) - all of the 73 patients, hemorrhagic syndrome - 15 cases; 11 cases had autoimmune manifestations. The treatment methods were: alfa interferon- 44 cases, Cladrarine - 12 cases, combined sevencial therapy with alfa interferon and Cladrarine - 8 cases, splenectomy - 8 cases, alk- lant agents - 5 cases. The response to the treatment was evaluated at 57 patients: 19 partial responses, 34 complete responses, 4 without response. 7 patients died, 3 because of severe toxico-septic shock. The complications in evolution were: infections(21 cases), hemorragic syndrome (2 cases), cardiovascular disease(2 patients); 2 patients achieved a second malignancy. Conclusion : HCL is an indolent lymphoproliferative disease, with well established diagnostic features (clinical, morphological, immunological), good response to the treatment, long remissions, but high incidence of infections, that appear at diagnosis or whenever through the evolution.

1408 DICHOTOMOUS MODEL TO EVALUATE TREATMENT OUTCOMES IN NON-HODGKIN’S LYMPHOMA PATIENTS
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Efficacy of treatment of malignant lymphomas is traditionally based on objective clinical data. At present patient-reported outcomes are of increasing importance to evaluate treatment outcomes in this patient population. Recently, the dichotomous model to evaluate treatment outcomes in patients with hematological malignancies was proposed by the experts of the EHA SWG “Quality of Life and Symptoms”. We aimed to test this model on new population of Non-Hodgkin’s lymphoma (NHL) patients receiving conventional chemotherapy (CT). 54 new NHL patients were enrolled in the study (stage IIb-IV, mean age 50.2 (SD 15.5), males/females - 55/59). The lymphoma histopathology was as follows: diffuse large B cell lymphoma - 24, peripheral T cell lymphoma - 11, anaplastic large cell lymphoma - 11, follicular lymphoma - 4, mantle cell lymphoma - 2, angioimmunoblastic T cell lymphoma - 2. All the patients underwent conventional CT: CHOP or CHOP-like regimens. QoL was assessed using the generic questionnaire SF-36. All the four patients required reduction of doses because of liver/toxicity, and one patient experienced infective toxicity (WHO grade 1-2). One patient was treated with only radiotherapy and two patients were observed because of indolent disease. After a median follow up of 51 months (range 1-128) from the end of therapy, 13 patients are alive and in complete remission; 7 patients died, 2 because of progressive disease and one patient died for progressive disease. Conclusions: Although the limited number of patients involved, and the peculiar subset of renal and liver allograft in which the risk of PTLD is low-intermediate, our study demonstrated that the majority of our patients developed aggressive NHL and an immuno-chemotherapeutic regime is feasible, well tolerated and allow to obtain an elevated percentage of remission.
1409
SALVAGE CHEMOTHERAPY WITH NON-PEGYLATED LIPOSOMAL DOXORUBICIN (ADRIAMYCIN), FLUDARABINE, OXALIPLATIN AND CYTARABINE (AFOXa) IN POOR-RISK B CELL NON-HODGKIN’S LYMPHOMA

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Objective: The aim of this study was to evaluate the feasibility and toxicity of the combination of non-pegylated liposomal doxorubicin (Adriamycin), fludarabine, oxaliplatin and cytarabine (AFOXa) in patients with diffuse large B cell lymphoma (DLBCL) in second or refractory relapse and the observed toxicity (MTD) seems to be MTD. 7 pts. (35%) achieved complete remission, and 13 (65%) of the 20 pts. with refractory disease (n=22), refractory relapse (n=1) and second (n=5) or third (n=1) relapse were enrolled. The intense pretreatment contained a median of 6 (range 1-18) cycles of chemotherapy. 25/29 pts. were pretreated with the anti-CD20 monoclonal antibody Rituximab. The AFOXa regimen consisted of non-pegylated liposomal doxorubicin (25 mg/m², days 1 + 3), fludarabine (25 mg/m², days 1-4), oxaliplatin (escalating doses of 100 or 130 mg/m², day 5) and cytarabine (escalating doses of 1000 or 1250 or 1500 mg/m², day 5). In the phase I part of the study (n=12) the maximal tolerable dose (MTD) was determined for oxaliplatin and cytarabine according to World Health Organization Common Toxicity Criteria (CTC). The primary objective of the subsequent phase II part of the study, which uses the determined MTD, is efficacy. Results: In the phase I part we established the MTD for oxaliplatin with 130 mg/m² and for cytarabine with 1000 mg/m². Out of 29 pts. 20 pts. were treated with the established MTD. 7 pts. (35%) achieved complete remission (CR or CRu) and 1 patient (5%) partial remission, with an overall response (OR) rate of 40%. A successful peripheral blood stem cell harvest through mobilization with the AFOXa regimen was possible in 10 pts. (50%). Conclusions: AFOXa is a feasible salvage protocol for patients with poor-risk recurrent or refractory NHL. The observed toxicity (MTD) seems to be acceptable considering the unfavourable prognosis and intensive pretreatment. The efficacy will be evaluated in the ongoing phase II study.

1410
INTENSIVE THERAPY OF THE PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA WITH PRIMARY INVOLVEMENT OF MEDIASTINAL LYMPH NODES AND PRIMARY MEDIASTINAL B-CELL LYMPHOMA EFFICACY AND TOXICITY

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Background. Primary mediastinal B-cell lymphoma (PMBCL) and diffuse large B-cell lymphoma (DLBCL) with primary involvement of mediastinal lymph nodes have different origins and, therefore, different immunological, molecular and genetic characteristics and response to therapy. Aims. To evaluate the efficacy and toxicity of the modified program NHL-BFM-90 (mNHL-BFM-90) for adult patients with PMBCL and DLBCL diagnosis and primary involvement of mediastinal lymph nodes. Results. 57 patients with large B-cell lymphoma with primary involvement of mediastinal lymph nodes. The illness was diagnosed in accordance with WHO classification criteria, the differential diagnostics of PMBCL and DLBCL was performed on the basis of the data obtained via immunochemical study through and assessment of the gene expression level by FCR. The diagnosis of DLBCL with primary involvement of mediastinal lymph nodes was established for 17 patients (7 men, 10 women, mean age 31 years (from 21 to 70 years)). All patients were treated according to mNHL-BFM-90 program in the Russian Hematological Research Centre between November 2004 and July 2010. DLBCL and PMBCL staging criteria developed by Ann Arbor were used for patients. All patients were diagnosed with II stage. Bulky mediastinal disease was found in 16 (94%) patients with DLBCL and in 19 (95%) patients with PMBCL. Serum lactate dehydrogenase level was increased in 17 (100%) patients with DLBCL and in 19 (95%) patients with PMBCL. ECOG 1 was established in 1 patient (2,7%), 2 - in 7 (18,9%), 3 - in 25 (62,1%), 4 - in 6 (16,7%) patients. The NHL-BFM-90 protocol was modified by us (a dose of methotrexate was reduced to 1500 mg/m² (12 h) in course A and B, doxorubicin (50 mg/m²) was included in the course A). 4-6 courses were performed, their quantity being determined depending on achieving the remission time. The patients with residual tumor in mediastinum underwent radiotherapy as consolidating treatment in total dose of 50 Gray. Results. 16 (94%) of the patients with PMBCL with primary involvement of mediastinal lymph nodes achieved a complete remission, and 13 (65%) of the patients with PMBCL. One of the patients DLBCL has died from chemotherapy complications. One patient with PMBCL turned out to be primary-resistant. Early relapse was ascertained in 1 patient with DLBCL and 3 with PMBCL. The 3-year disease-free survival (DFS) and overall survival (OS) were similar, 94% in patients with DLBCL and 84% with PMBCL. Most infectious and hemorrhagic complications occurred during the first course (course A), which can be explained by the initial poor condition of the patients and a large tumor mass at the start of treatment. Summary/conclusions. The modified mNHL-BFM-90 is a highly effective protocol. The 3-year DFS and OS were similar, 94% in patients with DLBCL and 84% with PMBCL.
Screening for Primary Immunodeficiency in Pediatric Patients with Lymphoid Malignancies

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Background. Primary Immunodeficiency diseases (PIDs), although rare in the general population are the best characterized and strongest known risk factors for lymphoid malignancies. Clinical immunodeficiency related score (IDR) is a recently introduced tool to identify possible patients with PID. Aim of the work is to evaluate the possibility of primary immunodeficiency in children with lymphoid malignancies by applying the clinical scoring tool and studying the humoral and cellular immune status at diagnosis, Patients and methods. We studied sixty two pediatric patients diagnosed as non Hodgkin lymphoma (NHL)(n=26), Hodgkin disease (HD)(n=16) and acute lymphoblastic leukaemia (ALL)(n=20). They were 41 males and 21 females aged 0.15-18 years. They were recruited from Ain Shams University, Children’s Hospital and National Cancer Institute in Egypt. Evaluation included a thorough history of previous illness followed by calculation of an immunodeficiency related score (IDR). IDR score ≥6 was suspicious of PID and IDR score ≥8 was highly suggestive of PID. Laboratory testing was performed in thirty two patients prior to start of chemotherapy, complete blood picture, immunoglobulin (Ig) A,M,G assay by nephelometry and quantification of T cell subsets by flowcytometry. Results: IDR score ≥8 was found in 2 ALL(10%), 7 NHL(27%) and 6(37.5%) HL patients(p=0.08), while IDR ≥8 was found in 4 NHL(15%),3 HL(18.8%) and none of ALL patients(p=0.58). IgG was higher in ALL 90% compared to HD 57.5% and NHL 33.3%(p=0.03). IgM level was lower in HD 50% compared to NHL 12.5% and ALL 0%(p=0.04), while no significant difference in IgA Level (p=0.25). ALL had the highest peripheral T cell subset levels. CD3 and CD8 levels were significantly low in HD (66.7%-66.7%) compared to NHL (12.5%-12.5%) and ALL (40%-20%) (p=0.05) respectively while CD4 levels were comparable (p=0.25). IDR score ≥9 was associated with low IgM level (p=0.05) but not other lgs or T cell subsets levels. Three patients with IDR score more than 8 were diagnosed as ataxia telangiectasia after their malignancy diagnosis (one Burkitt lymphoma, two HL) and one patient had leucocyte adhesion defect diagnosed prior to development of NHL (Burkitt lymphoma). Conclusions. Immune deregulation either raised lgs and peripheral T cell subsets due to chronic immunostimulation or decreased immune parameters as part of PID is associated with lymphoid malignancies. Creating a scoring system for screening of PID at diagnosis of lymphoid malignancy may be a useful tool for case detection and specific therapy.

Risk of Relapse in 81 Patients with Primary Nodal, Localised (I-II Stage) Diffuse Large B-Cell Lymphoma (DLBCL) in 1st Complete Response

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Background. DLBCL is an aggressive and potentially curable lymphoma. It presents itself as a localized disease in 80% of all cases and as primary nodal in 50-60% of these. Is known that in advanced disease, one third of them eventually relapse, but few data exist regarding localized disease. Aims. To analyse the risk and the characteristics of relapse of patients with primary nodal localized DLBCL in complete response (CR). Methods. Eighty one patient (43/58 M/F; median age, 59 years) in CR after chemotherapy, mainly consisting anthracycline containing regimen, were included in the study. Main clinicobiological characteristics at diagnosis and at relapse were analysed. Uni and multivariate studies were performed. Results. Seventeen patients (21%) eventually relapsed. Late relapse, more than 2 years after CR, were 6 patients, and early relapse, less than 2 years after diagnosis were documented in 11 patients. The most important variables predicting relapse at diagnosis were age and being it the only predictive variable in the multivariate analysis. No differences were found according to the treatment given and if they received regimen with or without rituximab. The second CR rate obtained in the patients after salvage therapies is a clear indication that in early relapsing (27% vs 50%). Median time from diagnosis to relapse was 1 year for patients for early relapsing and 4.5 years for late relapsing. Five-year overall survival (OS) was 18% for early relapsing patients and 83% for late relapsing patients (p=0.012). For DLBCL relapse, two-year OS was 50% versus 18% with autologous transplantation or not, respectively (p=0.079).

Gastrointestinal (GI) Tract Involvement in Advanced-Stage Diffuse Large B-Cell Lymphomas (DLBCL)

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Background and aims. The differentiation between primary extranodal GI tract lymphoma and secondary GI tract involvement in generalized lymphoma is difficult in many clinical situations but has a significant meaning while choosing treatment methods and strategies. The aims of this report is an attempt to characterize patients with GI tract involvement in advanced-stage DLBCL and indicating clues for treatment optimization. Materials and methods. We performed a retrospective analysis of 62 patients with CS IIB-IVB DLBCL treated in Chemotherapy Clinic in 2003-2009 regarding involvement of GI tract structures by lymphoma process. Such changes were observed in 9 patients. The group consisted of 5 women and 4 men aged 51-76 (median-58). CS has been evaluated as II-B (but multifocal nodal involvement in abdomen and pelvis)-3 patients, III-A-2 patients, III-B-1 patient, and IV-B-3 patients. After standard diagnostic procedures patients were qualified for ICHT according to R-CHOP scheme (6-8 cycles). The following factors were considered in the analysis: 1) localization and characteristics of GI tract involvement, 2) molecular DLBCL subtype: ABC or GBC, 3) clinical parameters: gender, age, symptoms, IPDI > 3, β2-microglobulin > 2.2 mg/L, 4) efficacy of surgical treatment if previously performed, 5) treatment efficacy understood as response to chemotherapy (CR/PR/SD/PD) and its duration (PFS/OS). Results: 1) the infiltration of stomach was found in 4 subjects, large intestine-3 subjects, small intestine-1 subject, a bifoical infiltration of small and large intestine-1 subject. The infiltration extended across the entire GI tract wall to local lymph nodes or adjacent lymphatic tissue in 6 patients, however in as many as 3 patients it did not cross GI tract serosa. 2) molecular subtype ABC has been diagnosed in 3 cases, GBC-5 cases, in 1 case the molecular subtype has not been tested. 3) the incidence of systemic symptoms-66% has been comparable to that in the overall DLBCL group-61%, similarly mean IPDI (3.2±3.1). LDH and β2- microglobuline. The only parameters that differed significantly between the groups were the incidence of anemia-56% in the group with GI tract involvement vs 35%, and need of RBC transfusions- 33% vs 14%. 4) surgical treatment preceded ICHT in 4 cases, In 3 patients lymphomatous infiltration has been found at the site of section or close to it during control enoscopy performed between surgery and ICHT. 5) CR after ICHT (mean 7-1 cycles) has been achieved in 5 cases (55% vs 69% in the overall DLBCL group), in 3 patients with PR a radioimmunotherapy with ibritumomab has been used as consolidation resulting in 1 CR +IPR+IPD ( death during further treatment ). Death of 1 patient caused by GD during ICHT. A 2-years-long PFS has been achieved by 5/9 (55%) while in the overall DLBCL group by 48/62 (69%). A 2-years-long OS for patients with GI tract involvement was 6/9 (67%) vs 48/62 (78%) in the overall DLBCL group. Conclusions. In advanced-stage DLBCL GI tract involvement is a negative prognostic factor for good ICHT R-CHOP response as well as its duration. Consolidation with ibritumomab in patients with PR only slightly improves treatment outcome.
RITUXIMAB-TREATMENT FOR RECURRENT ACQUIRED ANGIOEDEMA UNDERLYING LYMPHOPROLIFERATIVE DISORDERS: TWO CASE REPORT


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Background: Acquired Angioedema (AAE) due to the C1-inhibitor deficiency is often the Spy of an underlying autoimmune disorder or a lymphoproliferative disease (LPD). In these cases the acute symptoms can be resolved by the infusion of C1-inhibitor factor. Corticosteroids as immediate and etiological therapy in many cases is incapable of reducing the intensity and frequency of the AAE attacks. Aims: To obtain a faster and deeper control of AAE attacks and to avoid interferences of chemotherapy in their instable Complement’s Cascade, two Non Hodgkin Lymphoma (NHL) patients, refractory to corticosteroid therapy, were treated with Rituximab at 375 mg/mq weekly. CaseA: A 50 years old man with a history of recurrent AAE received, two years later, the diagnosis of Diffuse Large B Cell Lymphoma (DLBCL) from the biopsy of laterocervical lymph node with a presentation at stage III of disease. The C1-Inhibitor level was 4.8 mg/dl and functional C1-INH percentage was 5% without demonstrable antibodies against the C1-inhibitor (C1Gq=91mg/L). At moment of DLBCL diagnosis the patient presented weekly severe attacks of AAE without any benefit from corticosteroid therapy (Methylprednisolone 2mg/Kg BW). Due to progressive symptoms, we administered Rituximab at 375 mg/mq weekly for 4 weeks. After the 3rd dose the AAE attacks disappeared and C1-Inhibitor level and functional C1-INH percentage were 33,5 mg/dl and 45% respectively. Subsequently the patient received chemotherapy according to CHOP like scheme (Cyclophosphamide, Liposomal Pegilated Doxorubicin, Vincristine Prednisone) every 21 days for 6 doses and obtained a Complete Remission (CR). After one year the patient died due to acute myocardial infarction in CR from NHL and AAE. Case B: a 75 years old man with a recurrent AAE and a monoclonal gammopathy (0.98 mg/dl of IgG monoclonal component) presented a 40% of bone marrow involvement as unique manifestation by follicular NHL. C1-Inhibitor level was 6,4 mg/dl and functional C1-INH was 18%. No antibody against C1-inhibitor was present (C1Q 116 mg/L). The AAE attacks (2-3 monthly) were refractory to corticosteroid therapy. Rituximab was administered at standard dosage weekly for 4 weeks and every 3 months for 1 year as maintenance therapy. After the 3rd dose the patient obtained a remission from AAE with C1-Inhibitor level 30mg/dl and functional C1-INH 44% and, after 8th dose NHL marrow involvement disappeared. The patient is still in CR for AAE and NHL, after 1 year from the end of maintenance therapy. Conclusion: Our experience shows the efficacy of Rituximab in the treatment of AAE LPD-related in patients refractory to corticosteroid therapy even in absence of antibodies against C1-Inhibitor.

RITUXIMAB PLUS LIPOSOMAL PEGYLATED DOXORUBICIN IN PRIMARY CUTANEOUS B-CELL LYMPHOMAS

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Background. The most common types of primary cutaneous B-cell lymphomas (PCBCL) are follicle center cell lymphoma (FCL), marginal zone B-cell lymphoma (MZL) and diffuse large B-cell lymphoma of the leg (DLBCL-IT). They represent approximately one third of all cutaneous lymphomas. FCL and MZL generally have an indolent behavior and an excellent prognosis (>90% 5-year survival), different from DLBCL-IT (<60% 5-year survival). According to the EORTC/ESLCL recommendations, the first line treatment of FCL and MZL is most often radiotherapy (surgery in a minority of cases with a single skin lesion), while patients with multifocal skin involvement deserve a systemic antiblastic treatment (similar to those with relapsed/refractory disease). The option of first-line chemotherapy, viceversa, is the rule in DLBCL-IT. CHOP-like regimens are by far the most commonly used, although hard to propose in patients over 80, who are frequent in this subset. In this regard, the association of rituximab (R) and liposomal pegylated doxorubicin (PLD) appear very promising. Aims: Based on the favourable results reported with R and PLD in several recent trials, we decided to test efficacy and safety profile of this combination in patients with PCBCL. Methodology. From January 2005 to January 2010, 11 patients with PCBCL were treated with R plus PLD at the hematological divisions of Siena and Florence. Five of them were males and six females, with a median age of 56 years (range 39-81). Four patients had FCL, 4 MZL, and 3 DLBCL-IT. Ten patients had a stage II-III disease, while a female patient had a stage I disease with facial localization, and refused surgery and radiotherapy due to the high risk of aesthetic damage. Seven of 11 patients had relapsed after previous radiation therapy (5), chemotherapy (2) and chemotherapy (2). Treatment plan consisted of 2 monthly cycles of R 75mg/m2 and PLD 20mg/m2 d 1;15, followed (in responders) by two cycles at same dosage given only at day 1. All patients received prophylactic acetaminophen, chlorphenamine, low dose steroids and pyridoxine to prevent rituximab infusional side-effects and Palmar-Plantar Erythrodyssesthesia (PPE), respectively. Results: Ten out of eleven patients had a response (8 complete, CR; 2 partial, PR), while a patient did not respond (progressive disease, PD). All patients except two are alive and in stable remission, with a median follow-up of 24 months. One patient received a second cycle of progressive disease, and one due to a second neoplasm. Hematological toxicity was negligible (1 case of grade 2 neutropenia), as well as extra-hematological toxicity (2 cases of grade 2 PPE). Mild rituximab infusional reactions were registered in 3 cases at first infusion. Conclusions. These preliminary data suggest that R-PLD is effective and well tolerated in the treatment of all subset of PCBCL. Moreover, this therapeutic option may be offered front-line in case of peculiar localization, in which radiotherapy or surgery may cause permanent aesthetic damage.

HYPOALBUMINEMIA AS THE MOST SIGNIFICANT PREDICTOR OF POOR OVERALL SURVIVAL IN PATIENTS WITH MUCOSA-ASSOCIATED LYMPHOID TISSUE NON-HODGKIN LYMPHOMA

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Background. Mucosa-associated lymphoid tissue lymphoma (MALT) non Hodgkin lymphoma (NHL) belongs to the group of B cell non-Hodgkin’s lymphoma (NHL), a subgroup of indolent lymphoma. The latest scientific papers on MALT lymphoma are aimed at finding both the clinical and laboratory as well as the biological parameters which would initially, before starting the treatment, isolate the group of patients at a high risk of rapid disease progression. Aims. The aims of this study were to investigate prognostic clinical and laboratory factors significant for outcome of patients with MALT lymphoma, as well as to compared these factors between patients with gastrointestinal (GIT) and nongastrointestinal (non-GIT) sites of primary lymphoma. Methods. This study involved 87 patients with diagnosis of MALT lymphoma. The followed pretreatment laboratory parameters were recorded: hemoglobin, serum albumin level, serum lactate dehydrogenase level (LDH), beta2-microglobulin (B2-M), virologic (HBsAg, HCV, HIV) and bacteriological (Helicobacter pylori) status. Estimated clinical features were: stage of disease (CS) according Ann Arbor classification, performance status (PS) evaluated by the European Cooperative Oncology group (ECOG) recommendation, tumor mass voluminosity, number of extranodal localizations, laboratory as well as the biological parameters which would initially, before starting the treatment, isolate the group of patients at a high risk of rapid disease progression. Results: Ten out of eleven patients had a response (8 complete, CR; 2 partial, PR), while a patient did not respond (progressive disease, PD). All patients except two are alive and in stable remission, with a median follow-up of 24 months. One patient received a second cycle of progressive disease, and one due to a second neoplasm. Hematological toxicity was negligible (1 case of grade 2 neutropenia), as well as extra-hematological toxicity (2 cases of grade 2 PPE). Mild rituximab infusional reactions were registered in 3 cases at first infusion. Conclusions. These preliminary data suggest that R-PLD is effective and well tolerated in the treatment of all subset of PCBCL. Moreover, this therapeutic option may be offered front-line in case of peculiar localization, in which radiotherapy or surgery may cause permanent aesthetic damage.
MANAGEMENT OF GASTRIC LYMPHOMAS: METAANALYSIS OF also decreased serum albumin level. 

The multivariate analysis indicated the decreased serum albumin level to be the most significant predictor of poor OS: p=0.001, relative risk (RR)=5.060 (95% CI 2.055-12.458). In the group of patients with GIT localization of MALT lymphoma, the most significant predictor of poor OS was serum LDH level: p=0.081 (RR)=3.542 (95% CI 1.121-10.630), while the most significant predictor of poor OS in the group with non-GIT localization of MALT lymphoma was the decreased serum albumin level: p=0.001, (RR)=28.195 (95% CI 3.590^2 211,456). Conclusions: It is shown that MALT lymphomas with non GIT localization have longer OS in compare with GIT localization.

3,590^2 221,456). Conclusions: It is shown that MALT lymphomas with non GIT localization have longer OS in compare with GIT localization.

The most significant predictor of poor OS was decreased serum albumin level. At patients with GIT localization of MALT lymphoma, the most significant predictor of poor OS was serum LDH level, while at patients with non-GIT localization the most significant predictor of poor OS was also decreased serum albumin level.

**1418 NON-HODGKIN LYMPHOMA OF OCULAR ADNEXAL SITES SUBTYPED ACCORDING TO THE REAL CLASSIFICATION: A 17-YEAR EXPERIENCE FROM SINGLE INSTITUTION**

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**Background.** The Revised European and American Lymphoma (REAL) classification (1994) provided a useful and practical tool to define lymphomas. Aims. To report our experience with this unusual malignancy diagnosed since the REAL classification was implemented. Methods: Retrospective review was performed on patients (pts) with ocular adnexal non-Hodgkin lymphoma sites between 1994 and 2011. Medical records, diagnostic imaging procedures, and pathology reports, including immunophenotypes, were reviewed. Histologic definition applied the REAL Classification. Results. 10 pts were identified. All but one were females. Median age at diagnosis was 69 years (54-84). Primary sites included conjunctiva (5), orbital soft tissue (5), eyelid (2). No lacrimal gland location was found. Ann Arbor stage at diagnosis was IE in most cases (7). One patient (pt) had stage IIIE (conjunctival and maxillary sinus) and 2 pts had simultaneous bone marrow involvement (stage IV). 6 pts had marginal zone B-cell subtype, and the remaining 4 pts had small lymphocytic B-cell lymphoma. Radiation as single treatment modality was given to all 7 pts with stage IE, and all those achieved a complete remission (CR). CR was also achieved using chemotherapy with cyclophosphamide (C), vincristine (V), doxorubicin, prednisone (P), and rituximab followed by radiation in the pt with stage IIIE. Of the 2 pts with stage IV, 1 achieved a CR with rituximab-based chemotherapy, and 1 never achieved a CR despite chemotherapy (chlorambucil, CV) With a median follow-up of 25 months (9-70), the 9 pts that achieved an initial CR remain alive and disease-free. The pt that did not achieve a CR lost follow-up at 15 months. Conclusions. Radiation alone appeared to be an effective treatment modality for stage IE. Achieving a CR seems to be a crucial factor for long term control of the disease.

**1419 ROLE OF THE ENDOSCOPIC ULTRASONOGRAPHY IN THE MANAGEMENT OF GASTRIC LYMPHOMAS: METANALYSIS OF LITERATURE**

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**Background.** After Sackmann et al, in 1997 introduced the concept that early stage PGL responds to eradication treatment, the last decade has been characterized by a progressive increase in the use of endoscopic ultrasound (EUS) in staging and follow-up procedures. Results. STAGING: Despite first publications on the reliability of EUS in staging of PGL raised during the early 90’s (Caletti et al, 1993) reported an accuracy of 80-92% and 77-90% for T and N stage respectively, these data were not confirmed by successive studies (Fischbach et al, 2002), reporting an accuracy of 59% and 71% for T and N stage respectively, due to the fact that EUS is an operator-dependant technique (Janssens, 2009). Nevertheless, EUS has entered in the clinical practice of staging evaluation (Pavlovic et al, 2005; Di Raimondo et al, 2007) and has been indicated as the most appropriate technique in defining the loco-regional staging. FOLLOW-UP: Basically, there is a lack of studies concerning follow-up. Early reports with small series indicated a role of EUS both for staging and follow-up (Pavlick et al, 1997; Levy et al, 1997). More recent reports, however, have shown that endosonographic remission is documented with a significant delay respect to histology (Pöysä et al, 2002), even if at the same time another study confirmed that EUS is a dependable tool, finding a concordance between EUS and endoscopic biopsies of about 80% (Lagering et al, 2004), afterward, two reports stated that EUS is reliable in determining the response evaluation and the detection of disease reappearance (Yeh et al, 2003; Hoepffner et al, 2003). Lately, the Serbian group, found a stringent correlation between EUS and histology in both patients treated with HR-eradicating therapy and/or chemotherapy ± radiotherapy. Afterwards, our group carried out a retrospective study in our patients observed in the last 10 years in order to compare EUS with conventional endoscopy, confirming the aforementioned studies, cause EUS was concordant with biopsies in a small portion of patients, about one third. In addition, the EUS findings returned to normal with a considerable delayed time in respect to gastroscopy with biopsy, even after a prolonged follow-up. It is noteworthy that the endoscopic ultrasound (EUS) assay is of great help in determining an appropriate staging of the disease, but its role in follow-up lack of evidences and is not recommended.

**1420 PHASE I COMBINATION OF BORTEZOMIB AND EVEROLISMUS IN NHL: TOXICITY AND EARLY RESPONSE DATA**

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The mTOR/akt and NFkB pro-survival pathways contribute to pathogenesis of various NHLs. Preclinical data suggests the AKT/mTOR pathway is a potential target for drug discovery. Aims: To determine maximum tolerated dose (MTD), toxicity, and response rates. We present preliminary results. Results. MTD, toxicity, and response rates. We present preliminary results. All pts with relapsed/refractory NHL are eligible. A standard dose escalation design was employed. Pts continue study treatment until disease progression, elective withdrawal, or excess toxicity with no maximum number of cycles. B is given D1,4,8,11 of a 21 day cycle and everolimus continuously by mouth. The dose levels are: 1)B 0.7 mg/m² IV, E 5 mg PO QOD, 2) B 1 mg/m² IV, E 5 mg PO QOD, 3) B 1 mg/m² IV, E 5 mg PO QD, 4) B 1.5 mg/m² IV, E 5 mg PO QD, and 5) B 1.5 mg/m² IV, E 10 mg PO QD. 11 patients have been treated on study, with MCL (2 pts), Waldenstrom’s macroglobulinemia (1 pt), transformed FL (1 pt), DLBCL (2 patients), FL (4 pts), and CLL/SLL (1 pt). The transformed FL pt was later deemed ineligible and received 1 dose of drug. The median number of study cycles given is 3 (range 1-41). Treatment-related grade 3-4 toxicities included 1 instance of lymphopenia, thrombocytopenia, hyperglycemia, fatigue, hypophosphatemia, hyperkalemia, and syncope. In addition, grade 3 systolic heart
failure occurred in one patient after cycle 14, resolving on discontinuation of study drugs. The patient had no evidence of ischemic coronary disease on stress testing. Two other patients were removed from study at 2 and 4 cycles for adverse events possibly related to treatment, including grade 2 neuropathy and syncope/postural dizziness. Enrollment at the 3rd dose level proceeded; a DLT has been identified at that dose level. Responses are assessed using CT criteria every 3 cycles (9 weeks). 2 PR, 1 PD, 1 MCL (pts), 3 stable disease (2 FL, and one Waldenstrom’s), 4 progressive disease (2 DLBCL, 1 FL, 1 CLL/SLL), and 2 not evaluable (off study for adverse events prior to first response assessment) were observed. The Waldenstrom’s pt was treated for 41 cycles with radiographically stable disease and a decreasing paraprotein thought ultimately progressed after 29 months, leading to discontinuation. The MITD of B-E is 12 months and 17 months were diagnosed with stage IV disease. Enrollment proceeds at the 3rd dose level. B-E appears active in MCL and Waldenstrom’s but responses are not evident among other NHLs.

1421 CLINICAL AND MOLECULAR EVALUATION OF THE EFFICACY OF THE HIGH-DOSE POLYCHEMOTHERAPY IN ADULT PATIENTS WITH ANAPLASTIC LARGE-CELL ALK-POSITIVE LYMPHOMA

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Background. Anaplastic large-cell ALK-positive lymphoma (ALCL) - a disease which is characterized by an onset in young age, an aggressive course with high frequency of extranodal involvement (skin, subcutaneous fat, bone, lungs) and poor prognosis. The diagnosis is based on the biopsy findings. Gistological study revealed a diffuse growth of large anaplastic cells, which express T-cell markers, CD30 and ALK. In 80% of cases the disease is characterized by the translocation t (2;5) (p23; q35) and in other cases - by the variant translocations. The overall 5-year survival in adult patients is 40-60% (depending on the stage of the disease, type of chemotherapy and stem cell transplantation may be rational in patients with poor prognosis). The diagnosis is based on clinical and molecular prognostic factors. Hans classification that were statistically significant in univariate log rank comparisons of Kaplan-Meier survival curves were used to build a multivariable proportional hazard regression model of OS. Results. OS differed in 39,4% patients who also had anemia was only 3.1 years. A test for interaction of age and hemoglobin and age were also significant in a multivariate proportional hazard regression model. Low hemoglobin and increased age were independent prognostic factors. Hans classification of DLBCL need to be re-evaluated. Aims. To evaluate the relation of clinical and histopathologic characteristics to treatment outcomes in patients with DLBCL that were treated with rituximab-cyclophosphamide, adriamycin, vincristine, and prednisolone (R-CHOP) as first-line chemotherapy was investigated. Methods. Patients with newly diagnosed DLBCL treated with R-CHOP combination immunotherapy were retrospectively evaluated for their clinical characteristics, treatment efficacy and survival outcomes. Results. The overall survivals were analyzed. Results. 87 patients (median age, 57 years) were analyzed for the association of clinical characteristics to OS. Age >60 of the patients, elevated serum lactose dehydrogenase level, presence of 2 or more extranodal sites, and bone marrow involvement of DLBCL were powerful prognostic factors to OS in multivariate analysis. There was a significant difference of OS between the patients with CD20 expression and non-CD20 expression. Results of bcl-2 also failed to demonstrate the relation to OS. Whereas no difference enough for OS was shown between high-intermediate risk group and high risk group classified by the standard International Prognostic Index (IPI) (P=0.64), all 3 groups of revised IPI showed a clear-cut separation for EFS and OS. Conclusions. In the context of the current exploratory analysis, the revised IPI showed more clear-cut separation of patients according to their OS than the standard IPI, especially among patients that had more adverse clinical factors. Hans classification and the result of bcl-2 showed no predictive value for OS in patients with DLBCL treated with R-CHOP.
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CLINICAL CHARACTERISTICS, TREATMENT AND OUTCOME OF PRIMARY RECTAL LYMPHOMA: A SINGLE CENTER EXPERIENCE OF 16 PATIENTS
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Background. Rectum is a relatively uncommon site for lymphoma compared with other gastrointestinal sites and no consensus regarding management of primary rectal lymphoma (PRL) has been made due to its paucity. Aims. We aimed to investigate the clinical characteristics and treatment outcomes in PRL patients in a single center patient cohort. Methods. Between January 1998 and December 2010, 16 consecutive patients of PRL were identified and treated at the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients. Results. These 16 patients with PRL comprised 0.8% of all non-Hodgkin’s lymphoma patients (n=1984). The median age at diagnosis was 41 years (range, 30-66 years) and 11 were male. The most common presentations were anal bleeding (n=7) and abdominal pain (n=4) while 3 patients were asymptomatic. Eleven patients had stage IV disease by Ann Arbor staging. B-cell lymphoma (n=14) made up of majority of the series and half of them were extranodal marginal zone lymphoma (n=7). The others included 4 diffuse large B-cell lymphomas and 3 mantle cell lymphomas. Ten patients were given systemic chemotherapy with (n=5) or without rituximab (n=7) and 4 of them received additional local therapy including radiotherapy (n=5) or surgical resection (n=1). The others were treated with radiotherapy (n=5) or endoscopic mucosal resection (n=3) as a first-line therapy sparing systemic chemotherapy. Twelve patients (75%) achieved a complete response (CR) after first-line treatment. Especially all of those with extranodal marginal zone lymphoma (n=7) achieved CR after or after initial treatment, while 50% with the other histologic subtypes succeeded to gain CR. During median follow-up of 27.0 months (range, 2.8 - 123.5 months), 3 patients died and 4 patients experienced progression resulting in 2-year progression-free survival rate of 78.1% and a 2-year overall survival rate of 78.6%, and 4 patients experienced progression resulting in 2-year progression-free survival rate of 78.1% and a 2-year overall survival rate of 78.6%, respectively. Summary/Conclusions. PRL is very rare and seems to be mostly B-cell type. Extranodal marginal zone lymphoma may be one of the most common types of PRL with favorable treatment outcome.

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HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY WITH AND WITHOUT RITUXIMAB
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Background. Anti CD20 antibody (Rituximab) based chemotherapy regimens increase the HBV reactivation risk although sporadic HBV reactivation cases are reported in patients on maintenance with Rituximab single therapy too. We evaluated how many HBV reactivation occurred among patients Hepatitis B core antigen positive (HBcAB +) and Hepatitis B surface antigen (HBsAg) negative (HBsAg -) who received Rituximab single therapy and in 3 patients(1,9%) who had received chemotherapy including steroid and rituximab and in 3 patients(1,9%) who had received chemotherapy including regimen with only fludarabine without rituximab. Immediate administration of lamivudine therapy after elevation of HBV DNA level was conducted, and this resulted in reduction of it and improvement of liver function test. Conclusions. Rituximab plus steroid-containing regimens may increase the risk of HBV reactivation in HBsAg-negative and HBcAb-positive lymphoma patients but more attention should be paid on treatment with only fludarabine. More ambitious prospective studies are required to establish clinically useful or cost-effective follow-up methods for control of HBV reactivation in lymphoma patients with occult HBV infection.

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HEPATITIS B REACTIVATION IN PATIENTS WITH NON HODGKIN LYMPHOMA CD20+ UNDERGOING CHEMOTHERAPY WITH AND WITHOUT RITUXIMAB
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Background. Reactivation of HBV infection is a well-recognized complication in infected patients who undergo cytotoxic chemotherapy for cancer. The highest incidence of reactivation was reported in patients with non-Hodgkin’s lymphoma (NHL) and hematopoietic stem cell transplantation. Several case reports demonstrated that severe hepatotoxicity due to HBV reactivation after rituximab administration occurred both in hepatitis B surface antigen(HBsAg)-positive and HBsAg-negative patients. However, systematic evaluation of the relationship between HBV reactivation and rituximab is still limited. We conducted a study to investigate the relationship between rituximab-based therapy and HBV reactivation in 405 CD20-positive NHL patients at our institution. Methods. In our Unit, 405 CD20-positive NHL patients all newly diagnosed underwent measurement of HBsAg, anti-HBs, anti-HBc, HBsAg and anti-HBe. Patients were monitored by liver function tests during and after therapy as follows: on day 1 and day 14 of each cycle, every month for a year. Results. 154/405 (38%) patients were HBsAg-positive. 107 had an aggressive lymphoma, 42 had an indolent lymphoma. HBV reactivation was observed in 2 patients (1.2%) who had received chemotherapy including steroid and rituximab and in 3 patients (1.9%) who had received chemotherapy including regimen with only fludarabine without rituximab. Immediate administration of lamivudine therapy after elevation of HBV DNA level was conducted, and this resulted in reduction of it and improvement of liver function test. Conclusions. HBsAg and anti-HBe are very rare and seems to be mostly B-cell type. Extranodal marginal zone lymphoma may be one of the most common types of PRL with favorable treatment outcome.

1427
PERSISTENT B CELL POLYCLONAL LYMPHOCYTOSIS (PBL) WITH MASSIVE SPLENOMEGLAY MIMICKING MARGINAL ZONE LYMPHOMA
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Persistent polyclonal B-lymphocytosis (PBL) is a chronic expansion of polyclonal B lymphocytes seen predominantly in smoking women. Although considered as non-malignant it shares some features with malignant lymphomas like cytogenetic abnormalities and organomegaly. We report a case of PBL with massive splenomegaly indicated by polyclonal lymphocytes resembling splenic marginal zone lymphoma. Case report and methods. A 46 years old smoking woman was seen at our institution in June 2006 for lymphocytosis. Blood counts were WBC=15.8X109/L (lymphocytes=5.3% of WBC), Hb=100g/L and platelets=143X10E9/L. Serum biochemical analysis revealed a Ferritin level of 11mg/dl (N=20) and LDH=240U/L (N=160). IgM levels were increased (1696mg/dl N<=230), the rest of immunoglobulins were in the normal range and no M spike was apparent in serum proteinogram, immunofixation was also negative. Flow cytometry typing of lymphocytes by flow citometry revealed a B cell phenotype (CD19,CD22,CD20, positive for IgM, IgD, FMC7, CD27 and negative for CD5,CD10,CD23 with no light chain restriction. FISH analysis was negative for Chromosome 3 and 8 abnormalities. IgH rearrangement by PCR was polyclonal. The patient was positive for HLA DR. A CT scan revealed an enlarged spleen and small paraaortic lymph nodes. The patient developed progressive enlargement of the spleen measuring over 24cms with signs of portal hypertension and worsening of anemia and platelet level. Splenectomy was performed in April 2009. The spleen weighted 3.9 Kg. Histologically there was an extensive lymphoid infiltration from the marginal zone of the white pulp affecting also the red pulp. By Flow cytometry the lymphocytosis was in peripheral blood. By immunophenotyping the same lymphocytes were positive CNS and CD5,CD23, MNDA were positive and CD5, CD3, CD5, Bcl6, CD10, XBP1, CD23, CD43 negative without light chain restriction (Picture 1). Molec-
ular analysis showed polyclonal IgHV rearrangement. An isochromosome +1q (1q) was detected by FISH. After splenectomy the patient blood count return to normal range with no abnormal lymphocytes in the blood film. The presence of massive splenomegaly in patients diagnosed of PPBL has been recently recognized. To our knowledge only 6 cases including ours have been reported so far. All of them showed an histological pattern consistent with a marginal zone lymphoma but polyclonal in nature by immunophenotyping and molecular analysis. The biological mechanisms by which some patients with PPBL develop massive splenomegaly is unknown. Due to the rarity of this entity it should be kept in mind to avoid misdiagnosis.

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DISSECTING FOLLICULAR DENDRITIC CELL SARCOMA INTO SUB-RISK GROUPS ACCORDING TO CLINICAL OUTCOME

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Background. Follicular dendritic cell sarcoma(FDCS) is rare neoplasm and has been regarded as a low or intermediate-grade sarcoma. However, FDCSs show broad clinicopathological spectrum including some highly aggressive cases of short term survival. Currently, no well-established guideline is available for optimal treatment and predicting clinical outcome. Aims. To investigate clinicopathological factors predicting their clinical behavior and dissect them into sub-risk groups, and finally provide a base for optimal therapeutic planning, we analyzed 135 FDCSs with relevant clinicopathological parameters and clinical outcome. Methods: Among 34088 articles screened through Pubmed search (search terms; follicular dendritic cell tumor or follicular dendritic cell sarcoma or dendritic cell, 1986 to 2006), 71 articles which reported 135 FDCSs with available clinicopathological information were panned out. Repeatedly cited cases were carefully selected and adjusted to avoid the doubled data. Some missing pathological and clinical follow-up data were added by referring to original authors. 135 FDCSs were analyzed with relevant clinicopathological parameters including age, gender, location, tumor size, tumor margin, mitosis, nuclear atypism, hemorrhage, necrosis, Epstein-Barr Virus (EBV) association, Castleman’s disease association and treatment modality. For assessment of prognostic significance of each parameter, statistical analysis with Kaplan-Meier model and Cox-regression test were performed using SPSS, version 11.5. With combination of statistically validated parameters, we categorized FDCSs into three-tiered risk groups and analyzed their clinical outcome. Results: Mitotic Index (≥5/10 HPF) and intra-abdominal localization (P=0.0008) were significantly correlated with the clinical courses highly aggressive cases of short-term survival. Currently, no well-established guideline is available for optimal treatment and predicting clinical outcome. 6 FDCSs with well documented mitosis, tumor location and follow-up information were categorized into three groups; low risk(n=24, extra-abdominal location and mitosis ≤5/10HPF), intermediate risk(n=31, extra-abdominal location and mitosis ≤5/10HPF or intra-abdominal location and mitosis ≤5/10HPF) and high risk(n=11, intra-abdominal location and mitosis ≥5/10HPF). Three groups showed distinct clinical outcome in event-free survival (P=0.0000) and overall survival (P=0.0001). The median event-free survival were 96 months, 24 months and 11 months (low, intermediate and high risk group, respectively). None of low risk group died of disease (100% survival by 10 years), whereas high risk group showed aggressive clinical outcome (57.1% of 2-year survival). The intermediate risk group showed borderline clinical course (81.2%, 62.5% and 25% of 2-year, 5-year and 10-year survival, respectively). None of low risk group died of disease (100% survival by 10 years), whereas high risk group showed aggressive clinical outcome (57.1% of 2-year survival). The intermediate risk group showed borderline clinical course (81.2%, 62.5% and 25% of 2-year, 5-year and 10-year survival, respectively). Conclusions. Our results suggest that the FDCSs are very heterogeneous in clinical outcome and could be subclassified into three-tiered risk group system which would be useful for predicting prognosis and optimal patient management.

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NON-HODGKIN LYMPHOMAS IN CHILDREN WITH CHROMOSOME INSTABILITY SYNDROMES

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Background. Chromosomal instability syndromes (CIS) - a group of rare autosomal recessive diseases, which have common features of increased chromosome fragility phenomenon, immune deficiency, hypersensitivity to radiation and high predisposition to lymphoid tumour. CIS group include: ataxia-telangiectasia (AT), Nijmegen breakage syndrome (NBS), Bloom syndrome, Fanconi anemia and others. Methods. The diagnosis of NBS and AT is based on specific phenotype, results of clinical, laboratory and genetic investigations. The diagnosis of Non-Hodgkin lymphomas (NHL) is based of clinical, morphological and immunological researches of tumor substrate. Results. SS children with NHL were treated in the department of hematology, Lviv Children’s Hospital, during 1992-2009. 7 of them (8.2%) with CIS: 6 - with NHL (85.7%), 1 - with (14.5%). In 4 cases a diagnosis of CIS was established upon manifestation of oncological diseases in children, while in 3 children lymphoma developed during the follow up period. At the time of diagnosis of NHL median age of this children group was 9.3 years (4.3 years - 12.2 years). All patients with NBS and NHL were homoygous for the 657del5 NBS1 gene mutation. Children with NBS (85.7%), 1 - with (14.3%). In 4 cases a diagnosis of CIS was established upon manifestation of oncological diseases in children, while in 3 children lymphoma developed during the follow up period. At the time of diagnosis of NHL median age of this children group was 9.3 years (4.3 years - 12.2 years). All patients with NBS and NHL were homoygous for the 657del5 NBS1 gene mutation. Children with NBS had typical craniofacial abnormalities (microcephaly, ‘birdlike’ face) and short stature. The skin malformations (spots of hypo- and hyperpigmentation) were diagnosed in 3 children. The other typical detected defects: sindactyly and clynodaktyly (2 patients), renal hypoplasia (1 patient). Clinical picture of AT was comprised of progressive cerebellar manifestation: a peripheral group of lymph nodes and spleen - in 7 (100%) patients, abdominal lymph nodes - in 3 (24.9%) children, lesion of mediastinum with the compression syndrome - in 5 (71.4%) patients. Atypical cells (L1/L2-type lymphoblasts according to FAB-classification) in the bone marrow revealed in one (14.5%) boy with lymphoblastic lymphoma. Involved extranodal sites included liver (6 (85.7%) patients), lung tissue and pleura (4 (57.1%) patients), kidney (one (14.3%) child), bones (one (14.3%) girl). Treatment was conducted according to BFM-group protocols (NHL-BFM-95/NHL-DGLLU-2000). Two patients died prior to beginning and two at the first stages of chemotherapy from complications of tumour process and severe concomitant infections. 1 patient - as a result of tumour progression. Specific therapy was effective in 2 (28.6%) children which currently have a long-lasting remission (11 and 7 years). Summary. The severity of clinical course of NHL in patient with CIS is caused by infectious complications related to the background of combined immunodeficiency, which requires a powerful antibacterial, antifungal protection and correction of immune status. Cytostatic treatment of NHL in children with CIS is possible and should be attempted. Intensity of therapy should be adjusted to individual risk factors and tolerance.

1430

CAN WE CURE HTLV-I ASSOCIATED ADULT T CELL LEUKEMIA LYMHPHOMA?

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Background. Adult T cell leukemia (ATL) is one of the rare human cancers initiated by a transforming retrovirus, HTLV-I. After many years of
controversy, it is now accepted that the viral transactivator protein Tax plays a critical role in initiating the leukemic process, because Tax transgenics develop a disease with striking ATL features. However, its role in the maintenance of the lymphoproliferation remains still a matter of debate. Long-term prognosis of ATL patients remains extremely poor. In acute ATL, well conducted Japanese trials demonstrated that although combinations of chemotherapy improved response rate, they failed to achieve a significant impact on survival. Patients with chronic and smoking ATL have a better prognosis but long-term survival is poor when these patients are managed with a watchful-waiting policy or with chemotherapy.

**Aims and Methods.** We recently realized a worldwide meta-analysis. **Results.** We showing that the combination of zidovudine and interferon-alpha (IFN) is highly effective in the leukemic subtypes of ATL and should be considered as standard first line therapy in that setting. This combination has changed the natural history of the disease through achievement of significantly improved long-term survival in patients with smoldering and chronic ATL as well as patients with acute ATL and wild type p53. ATL lymphoma patients may benefit from initial induction therapy based on aggressive chemotherapy regimen in addition to or followed by antiretroviral therapy with AZT/IFN. In all patients, in order to prevent the occurrence of resistance and relapse, clinical trials assessing bone marrow transplantation additional targeted therapies such as arsenic/IFN combination or monoclonal antibodies, are mandatory after achieving CR. In that sense, we recently reported that the combination of arsenic trioxide, IFN, known to trigger Tax polyinosine in addition to zidovudine, yielded unprecedented response rates in chronic ATL patients, and may prevent relapses in ATL lymphoma responding patients after chemotherapy. To investigate the molecular mechanism of therapeutic action in vivo, we used Tax transgenic mice that develop a disease with striking ATL features. We demonstrate that the combination of arsenic trioxide and IFN cures Tax-driven murine ATLs through selective targeting of leukemia initiating cell (LIC) activity. Importantly, this effect requires proteasome function. Conclusions. Overall, our findings strongly suggest that in this model ATL LICs are addicted to the viral Tax oncprotein and open the prospect of new strategies to cure ATL.

**1431**

**DOSE ADJUSTED EPOCH - RITUXIMAB AS FIRST LINE TREATMENT FOR HIGH RISK, AGGRESSIVE B-NHL: A SINGLE CENTER EXPERIENCE**

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**Background.** Ose-adjusted EPOCH- rituximab (DA-EPOCH-R) is an infusional protocol (etoposide, vincristine, and doxorubicin) for 96 hours with bolus doses of cyclophosphamide and oral prednisone) which is based on pharmacodynamical adjustment of drug dosage depending on laboratory values of absolute neutrophil count and platelets (Wilson WH et al. Blood 2003;92:2685-2693). Rationale for this regimen is that tumors cells are less resistant on prolonged exposition to low doses of chemotherapy than to short exposition to high doses. Aims. To assess clinical outcome in patients with aggressive B-NHL with poor prognostic factors (very aggressive histology, IPI ≥2 and/or high proliferation index; Pl>80%, measured as percentage of Ki67+ cells) treated with DA-EPOCH-R in first line. Methods: From May 2005 to March 2011, 24 patients, median age 50.5 years (range 17-75) with poor prognosis, aggressive B-NHL, were included. Male/female ratio was 14/10.

Out of 24 patients 20 were DLBCL, 2 were Burkitt lymphomas and 2 were mantle cell lymphomas. Elevated LDH had 19 out of 24 patients (79%) and 18 out of 24 patients (75%) were in Ann Arbor stages 3 and 4. IPI ≥2 had 19 out of 24 patients (79%). DA-EPOCH-R was administered according to original schedule. Six of these patients proceeded to autologous hematopoietic stem cell transplantation (autoHSCT) as consolidation therapy due to very aggressive histology and biology of disease. In subgroup of 20 patients with DLBCL with median age of 55.5 years (range 17-75), 16 (80%) had elevated LDH, 16 (80%) were Ann Arbor 3 and 17 (85%) had IPI ≥2. Overall CR was 75% (18/24), including 14 (58%) complete responses (CR) and 4 (17%) partial responses (PR). Overall survival (OS) was 52% at 5 years (median not reached) with median follow up of 14 months (range 0-63). Of patients who achieved complete remission, 12 are still in CR with progression free survival (PFS) 75% at 5 years. In subgroup of patients with DLBCL 15 out of 20 patients responded to therapy (ORR 75%), 11 patients achieved CR (85%). Overall survival in this subgroup was 50% with median follow up of 11 months (range 0-63). Summary/Conclusions: In this report we included only patients who had aggressive B-NHL with poor prognostic factors. Our results are in line with previous report (Garcia-Suarez J et al. British Journal of Haematology 2009;147:276-283) who also find this regimen effective as first line treatment in high risk DLBCL patients. In certain subgroups of patients it may be option to continue treatment with autoHSCT as consolidation therapy. Only 2 patients relapsed during follow up period. All 6 patients who were transplanted are still in CR. When subgroup of patients with high risk DLBCL was analyzed separately, the results were similar as for entire group. We conclude that DA-EPOCH-R is highly effective regimen as first line treatment in high risk group of aggressive B-NHL.

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**OUTCOME OF CHRONIC LYMPHOPROLIFERATIVE DISORDERS ASSOCIATED WITH HEPATITIS VIRUSES INFECTIONS - SINGLE CENTER EXPERIENCE**

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**Background.** Chronic lymphoproliferative disorders (CLD) frequently associate hepatitis viruses infections. The role and timing of antiviral therapy remains to be defined - for HBV early therapy seems to prevent viral reactivation, for HCV the best strategy is not established. Aims. Analysis of hematological and virologic response after chemomunotherapy +/- antiviral. The hematological response was assessed using CT scan and bone marrow biopsy, the virologic response by determining quantitative viremia using TaqManPCR method. Results. We selected a group of 52 patients with CLD and hepatitis virus infection receiving therapy. HCV had 26/52(50%), 28/52(44.2%) HBV and 3 patients double/triple infection. Different histological types were found and divided into indolent/aggressive type: see Table 1. Chemotherapy +/-Rituximab was given in 19/52(36,53%) patients, 32/52(61,53%) received also antiviral therapy and one patient Interferon alone. In the group of patients receiving Rituximab representing 17/52 (32,69%), 11/17(64,7%) associated HBV and 6/17(35,3%) HCV. All HBV patients with R-chemotherapy associated antivirals. Four HCV patients received R-chemotherapy and antivirals. Interferon was administered in 13/52(25%) HCV patients and one HBV. Ribavirine+Interferon received 2 patients. Lamivudine was administered in 17/52(32,69%) patients and Entecavir in one. Complete hematologic response was assessed according to the indolent/aggressive type of CLD and HCV vs HBV - see table 2. A possible explanation is that HCV was mostly found in indolent types of CLD, responding better to therapy. Twenty six patients achieved complete response, most of them receiving chemotherapy and antiviral, 10 chemotherapy alone and one only Interferon.
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INTRAPLEURAL INSTILLATION OF RITUXIMAB FOR THE TREATMENT OF MALIGNANT PLEURAL EFFUSIONS IN CD20+ NON-HODGKIN’S LYMPHOMA

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Background. Malignant pleural effusion is a common clinical problem in patients with neoplastic disease. Approximately 10% of malignant pleural effusions are caused by Non-Hodgkin’s lymphoma (NHL). Aim. Four patients with NHL presented with unilateral pleural effusion, flow cytometry revealed CD19 and CD20 positive malignant cells. Two patients with follicular (grade 2) histological type of NHL (FL) had lifelong of effusion above two months. Two patients with diffuse large cell B-cell histological type of NHL (DLBCL) had lifetime of effusion less one month. Methods and results. Systemic chemotherapy (FCR or R-CHOP) and repeated percutaneous drainage were unable to control the effusion. Rituximab was instilled in a dose-escalating manner (starting dose 100 mg, maximal dose 400 mg) via the chest tubes into pleural spaces. The effusion resolved in both patients with DLBCL in two-three weeks. Complete remission was achieved in both DLBCL and FL patients.

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RITUXIMAB (RTX), METHOTREXATE (MTX) AND TEMOZOLOMIDE IS A SAFE OPTION FOR PATIENTS WITH PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA (PCNSL) AND POST TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD)

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Background. Although considered a curable form of lymphoma, PCNSL prognosis is still dismal. The backbone of treatment, the use of drugs with high cerebrospinal fluid (CSF) penetration such as MTX and cytarabine, can be toxic in the majority of patients, especially in the elderly. Therapeutic role of RTX is still not defined in these patients, and new drugs, like temozolomide are still under study. Aims: Describe the initial experience of a single institution in Brazil with a combined treatment with chemotherapy and immunotherapy in older or immunocompromised patients with PCNSL. Methods: From 2007 until now, all patients at our institution were treated following a regimen consisted of: RTX 100mg/m² D1-D5 and prednisone 120mg/m² every other day from D1 to D4. Intrathecal chemotherapy with MTX 15mg was delivered on D1, D5, D10 and D15. All patients received urine alkalization and folinic acid rescue after MTX treatment until MTX levels were <0.01 mmol/L. Patients achieving at least PR received five cycles of maintenance therapy, consisting of RTX and MTX 1g/m2 D1 and temozolomide 100mg/m² D1-D5 and prednisone 120mg/m² every other day from D1 to D45. Intrathecal chemotherapy with MTX 15mg was delivered on D1, D5, D10 and D15. All patients achieved complete hematologic responses, 72.2% (15/21) complete and partial responses (4) were achieved, but only one complete virologic response. Conclusions. Best hematological responses (complete and partial) were obtained in indolent types of CLD associated to HCV infection treated with combination of chemoimmunotherapy and antiviral therapy. The complete virologic response was revealed in patients with combined therapy, but a significant number of patients, associating antivirals still have detectable viremia, revealing that virologic response doesn’t parallel the hematological one. Still, adding antiviral therapy seems to be the best option as it prevented viral reactivation in most cases.

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BENDAMUSTINE IN THE TREATMENT OF RELAPSING AND REFRACTORY LYMPHOMA MALIGNANCIES: A SINGLE CENTER EXPERIENCE

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Background and Aims. Since 03-2009 we have had the opportunity to treat relapsing and refractory lymphoma patients with bendamustine. 29 patients with either follicular (5), mantle cell (2), marginal zone lymphoma (10), hairy cell leukemia (1), CLL (7), DLBCL (4) and Hodgkin’s lymphoma (2) were treated with this bifunctional alkylating agent. Median age of the 27 NHL patients was 56y with a M/F of 11/16. These patients were extensively pretreated (median 3 range 1-7). The 2 female Hodgkin patients were younger (25 and 30y) with 5 to 6 pretreatment regimens. Methods. Bendamustine was administered every 4 weeks for up to 6 cycles at a dose of 90 mg/m² days 1 and 2. For the CLL patients a dose of 70 mg/m² on days 1 and 2 was chosen. Bendamustine was added on day 1 of each cycle in 14 patients. Results: 17 patients (59%) received the intended 6 cycles of bendamustine. Early withdrawals were due to adverse events (n=2), disease progression (n=4) or patient decision (n=5). Dose reductions and treatment delays occurred respectively...
INCIDENCE OF GASTRIC INVOLVEMENT IN NON-GASTRICAL MARGINAL ZONE LYMPHOMA

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Background. There was an observation that a significant proportion of patients presenting with nongastrical marginal zone lymphoma (MZL) had gastric involvement as well, thus arguing for esophagogastroduodenoscopy (EGD) as routine diagnostic workup of extranodal MZL. However, the incidence of gastric involvement in nongastrical MZL has not been investigated in Asia, where the incidence of MZL is higher than that in Western countries. Aims. The present study was undertaken to assess the incidence of gastric involvement in nongastrical MZL.

Methods. Between April 1998 and December 2010, 153 consecutive patients with nongastrical MZL were treated in the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients.

Results. The median age at diagnosis was 51 years (range, 16 - 79 years), and male patients consisted of 42% (n=64). One hundred patients (86.3%) initially presented as localized disease (Ann Arbor stage I or II), 2 (1.3%), 15 (9.8%), 4 (2.6%) patients were stage IIIa, IVa, IVb, respectively. Most of the patients (n=136, 88.9%) had extranodal involvement of lymphoma at a single site. The most common extranodal sites were ocular, nasal (46.4%), intestine (11.1%), lung (9.8%) and nasal sinus (5.9%). Among those 153 patients, 47 (30.7%) had undergone EGD as part of their initial workup. None of these 47 patients was found to have gastric involvement of lymphoma. The most common endoscopic and pathologic finding of EGD was chronic superficial gastritis (n=16, 34%). Summary. None of the 47 patients who had undergone EGD had gastric involvement of EGD. Our findings do not support routine EGD in patients with extranodal MZL.

A MONOCENTRIC EXPERIENCE OF CASTLEMAN’S DISEASE

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Background. Castleman’s Disease (CD), is a rare and poorly understood disease characterized by a pathologic growth of lymphoid tissue. This disease is composed of two different type: unicentric and multicentric disease. Although the optimal therapy is unknown. We report our experience about patients with diagnosis of CD, seen at our Institution since 2004.

Methods and Aims: From January 2004 to December 2010 we observed 6 patients affected by CD (2 males, 4 female, median age 65, range 49-82) Unicentric disease was defined as a solitary mass; multicentric disease compromised patients with multiple masses. We evaluated the involvement of human herpesvirus 8 (HHV-8) in all patients. Clinical, radiologic and laboratory data were analyzed to evaluate treatment response. Results: Among 6 patients, 2 presented unicentric disease, 4 multicentric. All patients underwent lymphonodal biopsy and were so classified: 1 in plasma cell type, 4 hyaline-vascular, 1 mixed; the 2 patients with multicentric disease presented hyaline-vascular histology. At evaluation of HHV-8 infection, 2/6 resulted positive at diagnosis; this patients presented a multicentric disease with systemic symptoms. The patient with plasma cell type (a female of 50 years old) developed CD from 6 years of diagnosis of POEMS syndrome; she was treated with high dose cyclophosphamide and was planned for autologous stem cell transplant. The 2 patients with HHV-8 infection were treated with polichemotherapy, resulting resistant. After chemotherapy they were treated with oral valacyclovir, both obtaining a complete remission after 2 months of treatment and up to now they are in complete remission after 4 and 2 years respectively. The remaining 3 patients, the 2 monocentric disease underwent lymphonodal excision and the last one 1 (multicentric CD) was submitted only to diagnostic lymphonodal biopsy. These 3 patients remaining symptoms free without any other treatment from 7,1 and 2 years respectively Conclusions: In our series, the asymptomatic patients (either unicentric or multicentric) did not need any treatment. The 2 symptomatic patients failing chemotherapy and benefited for antiviral treatment. A longer follow up and a larger series are needed to evaluate the better therapy for either symptomatic or asymptomatic disease.

INCIDENCE OF GASTRIC INVOLVEMENT IN NON-GASTRICAL MARGINAL ZONE LYMPHOMA

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Background. There was an observation that a significant proportion of patients presenting with nongastrical marginal zone lymphoma (MZL) had gastric involvement as well, thus arguing for esophagogastroduodenoscopy (EGD) as routine diagnostic workup of extranodal MZL. However, the incidence of gastric involvement in nongastrical MZL has not been investigated in Asia, where the incidence of MZL is higher than that in Western countries. Aims. The present study was undertaken to assess the incidence of gastric involvement in nongastrical MZL.

Methods. Between April 1998 and December 2010, 153 consecutive patients with nongastrical MZL were treated in the Asan Medical Center, Seoul, Korea. We retrospectively analyzed the results of these patients.

Results. The median age at diagnosis was 51 years (range, 16 - 79 years), and male patients consisted of 42% (n=64). One hundred patients (86.3%) initially presented as localized disease (Ann Arbor stage I or II), 2 (1.3%), 15 (9.8%), 4 (2.6%) patients were stage IIIa, IVa, IVb, respectively. Most of the patients (n=136, 88.9%) had extranodal involvement of lymphoma at a single site. The most common extranodal sites were ocular, nasal (46.4%), intestine (11.1%), lung (9.8%) and nasal sinus (5.9%). Among those 153 patients, 47 (30.7%) had undergone EGD as part of their initial workup. None of these 47 patients was found to have gastric involvement of lymphoma. The most common endoscopic and pathologic finding of EGD was chronic superficial gastritis (n=16, 34%). Summary. None of the 47 patients who had undergone EGD had gastric involvement of EGD. Our findings do not support routine EGD in patients with extranodal MZL.

A MONOCENTRIC EXPERIENCE OF CASTLEMAN’S DISEASE

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Background. Castleman’s Disease (CD), is a rare and poorly understood disease characterized by a pathologic growth of lymphoid tissue. This disease is composed of two different type: unicentric and multicentric disease. Actually the optimal therapy is unknown. We report our experience about patients with diagnosis of CD, seen at our Institution since 2004.

Methods and Aims: From January 2004 to December 2010 we observed 6 patients affected by CD (2 males, 4 female, median age 65, range 49-82) Unicentric disease was defined as a solitary mass; multicentric disease compromised patients with multiple masses. We evaluated the involvement of human herpesvirus 8 (HHV-8) in all patients. Clinical, radiologic and laboratory data were analyzed to evaluate treatment response. Results: Among 6 patients, 2 presented unicentric disease, 4 multicentric. All patients underwent lymphonodal biopsy and were so classified: 1 in plasma cell type, 4 hyaline-vascular, 1 mixed; the 2 patients with multicentric disease presented hyaline-vascular histology. At evaluation of HHV-8 infection, 2/6 resulted positive at diagnosis; this patients presented a multicentric disease with systemic symptoms. The patient with plasma cell type (a female of 50 years old) developed CD from 6 years of diagnosis of POEMS syndrome; she was treated with high dose cyclophosphamide and was planned for autologous stem cell transplant. The 2 patients with HHV-8 infection were treated with polichemotherapy, resulting resistant. After chemotherapy they were treated with oral valacyclovir, both obtaining a complete remission after 2 months of treatment and up to now they are in complete remission after 4 and 2 years respectively. The remaining 3 patients, the 2 monocentric disease underwent lymphonodal excision and the last one 1 (multicentric CD) was submitted only to diagnostic lymphonodal biopsy. These 3 patients remaining symptoms free without any other treatment from 7,1 and 2 years respectively Conclusions: In our series, the asymptomatic patients (either unicentric or multicentric) did not need any treatment. The 2 symptomatic patients failing chemotherapy and benefited for antiviral treatment. A longer follow up and a larger series are needed to evaluate the better therapy for either symptomatic or asymptomatic disease.

MONITORING OF MINIMAL RESIDUAL DISEASE IN MANTLE CELL LYMPHOMA

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Background. Mantle cell lymphoma (MCL) is characterized by a specific chromosomal translocation t(11;14)(q13;q32) resulting in cyclin D1 over-expression and cell-cycle dysregulation. It is historically considered particularly through staging. The role of interim examinations are still challenging, further studies are needed to evaluate its exact role.

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molecular relapse enables an early therapeutic intervention (for example with immunotherapy) resulting maybe in a prolongation of the period without a clinical relapse.

**1440**

**RITUXIMAB PLUS BENDAMUSTINE (RB) REGIMEN IN ELDERLY PREVIOUSLY UNTREATED PATIENTS WITH INDOLENT, NON FOLLICULAR NON HODGKIN LYMPHOMA: PRELIMINARY DATA OF A SINGLE CENTRE STUDY**

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Background: Bendamustine (B), a byfunctional chemotherapeutic agent, has shown considerable activity for solid and lymphoid malignancies. B has recently become available for clinical use, as a first-line treatment for chronic lymphocytic leukaemia (CLL) and as salvage therapy after Rituximab or Rituximab-based regimen, for early relapsed or refractory indolent B-cell lymphoma (NHL). Recent clinical trials have found B to be well tolerated and effective, as first line treatment in CLL and as a treatment in relapsed or refractory indolent NHL. Aims: to assess the efficacy and toxicities of B in combination with Rituximab in elderly previously untreated patients (pts) with indolent, non follicular NHL. Methods: from October 2008 to May 2010, 20 pts (M/F=15/5) with previously untreated indolent, non follicular NHL were enrolled in the study. The median age was 74 yo (range: 64-85); seventeen pts (85%) were more than 70 yo and 11 (55%) had B-CLL/SLL, 3 (40%) LPL/WM an 5 (8%) SMZL. Eight pts (40%) present an ECOG PS > 1. ENS involvement was present in one (5%) and the BM involvement in all pts (100%). Fourteen (70%) pts had co-morbidity with more than 2 disease in 80% of cases. R-B regimen consisted of Rituximab 357.5 mg/mq iv on day 1 and Bendamustine 80 mg/mq iv on days 1, 2; all pts received six-eight cycles delivered every 21-26 days; the response assessment was planned after 3 cycles and at the end of treatment. Median number of cycles delivered was 5 (range 3-8); 14 pts (70%) completed the planned treatment; dose reduction occurs in 4 pts (20%). Nine pts (45%) received G-CSF at the dose of 500 mcg/die as primary (10%) and secondary (25%) prophylaxis. ESA support was needed in 4 (22%). Results: complete response (CR) was achieved in 11 (55%) and partial response (PR) in 9 (45%) pts with an ORR of 100%; no toxic death or relapses occur. Ten out of 11 (91%) pts with B-CLL/SLL achieved CR, while all pts with LPL/WM obtained stable RP. The regimen was safe and well tolerated with dose reduction occurring only in 4 pts (20%). The mainly adverse events recorded was neutropenia occurring in (39%); severe neutropenia (WHO grade 3-4) was recorded only in 4 pts (20%). No extra-hematological toxicity was observed. With a median follow-up of 16 months (range: 4-23) OS, PFS and RFS were 94% and 100% respectively. Conclusions: RB is an effective regimen for elderly pts with previously untreated indolent non follicular NHL, mainly in CLL/SLL. RB is a safe regimen with major but tolerable toxicities consisting in myelosuppression. A longer follow-up is needed to define response duration and long-term safety.

**1441**

**FDG PET CT SCAN ROLE ON DIAGNOSIS AND INITIAL STAGING OF LYMPHOPROLIFERATIVE DISEASES**

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Background: FDG PET CT scan (PET) was recently introduced as a part of initial staging of many lymphoproliferative diseases. PET is currently used during initial and restaging to detect disease sites which are not recognized with other clinical and laboratory methods. However, the role of PET on diagnosis and staging of lymphomas remains to be seen. Aim: To study the findings of PET during initial diagnosis of lymphoma patients, to compare them with the findings of the conventional staging methods and to examine the possible role of PET on initial staging of lymphoma, in respect to its subtype. Methods: From 2003-2010, 131 patients with recent diagnosis of lymphoma, underwent PET as part of their initial screening. All patients underwent conventional clinical and laboratory initial staging with CT scan and bone marrow biopsy (BMB) and were staged according to standard systems. A comparison between conventional vs PET findings and staging, including special sites of disease, ie spleen and bone marrow (BM), in relation with the discrete subtype of lymphoma, was made. Results: 131 patients performed PET during initial diagnosis of lymphoma in Hematology Clinics of University of Athens and Athens Medical School.

The lymphoma subtype was Hodgkin (HL) in 65 pts, follicular in 21 pts, DLBCL in 18 pts (8 primary mediastinal), and other lymphoproliferative diseases in 27 pts. The lymphoma subtype was Hodgkin (HL) in 65 pts, follicular in 21 pts, DLBCL in 18 pts (8 primary mediastinal), and other lymphoproliferative diseases in 27 pts. B-CLL/SLL were staged in 11 pts (91%), while all pts with LPL/WM obtained stable RP. The regimen was safe and well tolerated with dose reduction occurring only in 4 pts (20%). Nine pts (45%) received G-CSF at the dose of 500 mcg/die as primary (10%) and secondary (25%) prophylaxis. ESA support was needed in 4 (22%). Results: complete response (CR) was achieved in 11 (55%) and partial response (PR) in 9 (45%) pts with an ORR of 100%; no toxic death or relapses occur. Ten out of 11 (91%) pts with B-CLL/SLL achieved CR, while all pts with LPL/WM obtained stable RP. The regimen was safe and well tolerated with dose reduction occurring only in 4 pts (20%). The mainly adverse events recorded was neutropenia occurring in (39%); severe neutropenia (WHO grade 3-4) was recorded only in 4 pts (20%). No extra-hematological toxicity was observed. With a median follow-up of 16 months (range: 4-23) OS, PFS and RFS were 94% and 100% respectively. Conclusions: RB is an effective regimen for elderly pts with previously untreated indolent non follicular NHL, mainly in CLL/SLL. RB is a safe regimen with major but tolerable toxicities consisting in myelosuppression. A longer follow-up is needed to define response duration and long-term safety.

**Table 1. PET vs CT/BMB findings in lymphoma pts.**

The lymphoma subtype was Hodgkin (HL) in 65 pts, follicular in 21 pts, DLBCL in 18 pts (8 primary mediastinal), and other lymphoproliferative diseases in 27 pts. The lymphoma subtype was Hodgkin (HL) in 65 pts, follicular in 21 pts, DLBCL in 18 pts (8 primary mediastinal), and other lymphoproliferative diseases in 27 pts.

**DISCUSSION**

**DIFFERENT RISK FACTORS FOR RELAPSED FOLLICULAR LYMPHOMA TREATED WITH CHEMOTHERAPY VERSUS IMMUNOCHEMOTHERAPY**

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Background: All widely accepted prognostic indices in follicular lymphoma (FL) were primarily designed for patients with newly diagnosed disease. Nowadays, the precise assessment of risk also in relapsed FL patients seems to be necessary, since lot of treatment strategies are available for these patients. Aims: The aim of this study was to compare the outcome of FL patients in first relapse treated with chemotherapy and immunochemotherapy and to identify high risk patients in both groups. The second aim was to evaluate the impact of adding rituximab to chemotherapy on overcoming the negative prognostic impact of previously identified risk factors in patients treated with chemotherapy. Additionally, 6 pts (5%), all with HL, presented increased spleen uptake without splenomegaly on CT scan. Conclusion: PET confirms practically all CT findings, as far as lymphadenopathy is concerned and occasionally reveals additional involved sites. It also helps in defining splenic infiltration. It cannot frequently reveal bone marrow infiltration as compared to BMB. The histological subtype of disease should be taken under consideration for the interpretation of PET results. The clinical significance of PET contribution in staging of lymphomas should be further clarified, adding, in controversial cases, tissue biopsies.

**Table 1. PET vs CT/BMB findings in lymphoma pts.**
first relapse of FL grade I, II or IIIa, in the period February 2002-April 2009. In the first line, the patients were treated with R±CHOP or R±CVP. In the first relapse they were treated with fludarabine based regimens, 53 patients received immunchemotherapy (R-FC, R-FND) and 27 chemotherapy (FC, FND). The characteristics in first relapse as possible risk factors were age, higher histological grade in relapse, presence of “bulky” tumor (>10 cm in diameter), ECOG performance status (ECOG PS >1 and FLIPI high risk as the independent risk factors for patients treated with chemotherapy. Older age (>60 years) and presence of B symptoms in multivariate Cox regression analysis were identified as the independent risk factors for patients treated with immunochemo-therapy. In the comparative analysis, significantly longer overall survival after relapse was revealed in patients with ECOG PS ≥1 (log rank 12.679, p<0.01), FLIPI score ≥2 (log rank 14.712, p<0.01) and B symptoms (log rank 4.676, p<0.05) if they were treated with immunochemo-therapy. The improvement in survival after relapse by addition of rituximab was not recorded in elderly patients (log rank 0.001, p>0.05). Conclusions: Use of immunochemo-therapy in first relapse of FL brought to clear benefit in survival. Older age and presence of B symptoms are risk factors for poor outcome in patients treated with immunochemo-therapy. The addition of rituximab to chemotherapy overcame the negative prognostic impact of high FLIPI risk and poor ECOG PS of FL. Studies with large series of patients treated with immunochemo-therapy in relapsed FL are needed, in order to identify patients who maybe require more aggressive therapeutic approach.

1443 SUCCESSFUL TREATMENT WITH PREDNISONE ALONE FOR BING NEEL SYNDROME

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Background.Waldenstrom's macroglobulinemia (WM) is the result of clonal proliferation of lymphocytes that produce monoclonal immunoglobulin M. Many central nervous system (CNS) complications have been described, the majority associated with blood hyperviscosity syndrome. However, CNS infiltration by plasmacytoid lymphocytes (Bing -Neel syndrome) has only rarely been reported. We report two cases of diffuse Bing-Neel syndrome presentation effectively treated with low-intermediate dose prednisone. Case summary. Case 1. A 81-year old man was diagnosed with Waldenstrom's macroglobulinemia and progresive cognitive decline was admitted for seizure and coma in November 2003. Electroencephalogram showed mild diffuse cerebral dysfunction and moderate right frontotemporal slowing with epileptiform dis charges. Cerebrospinal fluid (CSF) examination was unremarkable. Cryoglobulin testing was negative. Computed tomography of the head showed enhancing heterogenous periventricular hypodensities. Phenytoin was started with sudden neurological improvement. In the following months however the patient showed progressive cognitive deterioration with dizziness, confusion and lethargy associated with persistent monoclonal immunoglobulin M. In May 2004 low dose prednisone was started with complete and durable mental restoration. Case 2. A 61 year old man diagnosed with WM was admitted for septic fever, haemolytic anaemia and mental deterioration in January 2010. He was started on steroid treatment with clinical and haematological improvement. In December 2010 due to the monoclonal immunoglobulin increase the patient received three weekly courses of Rituximab and was admitted with confusion and septic fever in December 2010. Despite of the septic state resolution the patient showed persistent cognitive decline with drowsiness. CSF examination revealed high protein concentration without cellular abnormalities. MRI of the brain showed left parietal white matter T2 hyperintensities. Intermediate dose steroid treatment (50 mg Prednisone) was started achieving remarkable clinical improvement, with persistent IgM concentration. Conclusions. Bing Neel syndrome is a rare and potentially treatable complication of WM. In patients presenting with rapidly progressive cerebral deterioration and monoclonal immunoglobulin M, Bing-Neel syndrome should be considered and effectively treated with corticosteroid therapy.

1444 PREVALENCE OF HAIRY CELL LEUKEMIA IN HUNGARY WITH SPECIAL EMPHASIS ON THE TREATMENT HABITS OF HUNGARIAN HEMATOLOGISTS

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Background. Hairy cell leukemia is a rare disease, it’s incidence in Hungary is 2 patients per million inhabitants. According to the international guidelines, the treatment of choice drugs in HCL are cladribine and pentostatin. Overall survival of treated patients does not differ significantly from that of the general population. Aim. To establish the consequence of treatment choices on the quality of hairy cell leukemia care. Methods: We have sent questionnaires on treatment habits of Hungarian hematologists in HCL and on the main characteristics the HCL patients they care for. All Hungarian haematological departments and ambulances responded enabling us to evaluate retrospectively the data of 180 HCL patients. Results: Seventy percent per cent of the patients were male, 24% were female. Average age at diagnosis was 57 years (range 34-92). First line treatment modalities were the following: interferon-alpha (76% of cases), cladribine (14%), splenectomy (5%), watchful waiting (6%). Cladribine was given as second line treatment in 56% of cases, while further second line treatment options applied were: alpha-interferon (32%), watchful waiting (31%), rituximab (7%), pentostatin (3%), splenectomy (1%). First line alpha-interferon was given for less than 3 months in 19 percent of cases, for 3 to 12 months in 33% of patients, while duration of first line alpha-interferon treatment was more than 12 months in 40% of cases. Looking at second line treatment 48% of patients received alpha-interferon for 3 to 12 months, while the percentage of patients receiving alpha-interferon for more than 12 months was even higher, 57% of cases. At the time of our survey 77% of the patients had non-symptomatic stable disease, 6% had non symptomatic progressive disease, 6% had symptomatic progressive disease and 4% had advanced symptomatic progressive disease. All three latter categories necessitating further treatment. Conclusions: Although cladribine treatment is cost-effective and one treatment cycle results in durable complete remission in 75-80% of cases, in Hungary long lasting interferon-alpha treatment is still widespread, because of the peculiar differences of financing alpha-interferon and cladribine. Cladribine, even the sc. form receives financing only for inpatients, at the primary expense of the hospital. Looking at our findings, earlier and more widespread use of cladribine seems to be justified both from the medical and from the phar-maco-economic point of view.

1445 PROGNOSTIC SIGNIFICANCE OF THE KI-67 INDEX IN DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS TREATED WITH RITUXIMAB PLUS CHOP - EXPERIENCE OF SERBIAN LYMPHOMA STUDY GROUP

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**Background.** Introduction of the anti-CD20 antibody, Rituximab, in therapy of patients with diffuse large B-cell lymphoma (DLBCL) significantly improved survival and altered the predictive values of known prognostic factors in DLBCL. Assessment of tumor cell proliferation based on Ki-67 expression yielded conflicting prognostic predictions of patients with diffuse large B-cell lymphoma (DLBCL). Aims. The aim of this study was to evaluate the role and prognostic significance of the Ki-67 proliferation index (PI) in DLBCL patients treated with rituximab plus CHOP (R-CHOP). METHODS: Ki-67 was assayed immunohistochemically in tissue samples of 145 patients with newly-diagnosed DLBCL treated with R-CHOP between January 2007 and January 2011. Results: The complete response (CR) rates following R-CHOP administration were not significantly different, based on Ki-67 expression status (RFS) rates were 82.2% in patients with a low index Ki-67 (Ki-67 ≤ 80%, n = 107) compared with 73.7% in patients with a high index (Ki-67 ≥ 80%, n = 38). In patients with a low IPI (≤ 2), one-year survival was 87.4% and 69.8% in those with a higher index (IPI > 2). In patients with diffuse large B-cell lymphoma Ki-67 PI of 80% was found to significantly discriminate patients with good or bad prognosis when combined with low IPI score (AUC 0.649, P = 0.013). In multivariate analysis, Ki-67 expression combined with low IPI score (≤ 2) was a significant prognostic factor for EFS [hazard ratio (HR) = 1.760; 95% confidence interval (CI) 1.030-3.008; P = 0.05]. Conclusions: In diffuse large B-cell lymphoma, a cut-off value of 80% can distinguish patients with a good and bad prognosis when combined with another prognostic factor as low IPI score.

**DIFFUSE LARGE B-CELL LYMPHOMA WITH HEPATITIS C VIRUS- INFECTION TREATED BY CHEMOTHERAPY WITH RITUXIMAB REGIMENS: PROGNOSIS AND TOXICITY**

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Many epidemiologic studies have demonstrated an association between hepatitis C virus (HCV) infection and non-Hodgkin lymphoma, suggesting that HCV plays a role in the development of this malignancy. In a number of researches it is shown that level of various markers of a hepatitis C at patients from NHL can be taped at 30% of patients. In our research we studied function of a liver at patients with NHL carrying out immunohistochemistry with markers of a hepatitis C and without them. 41 patients (pts) with NHL have been included in research with markers HCV infection and 108 without it by which it has been spent immunohistochemistry. The age median patients who were HCV-positive was 47 years. 30 patients HCV-positive have III-IV stages of disease and II stage. Normal level ALT and AST prior to the beginning of treatment was at 6 patients. The median serum level HCV RNA at the beginning of treatment was 2.3x10^7/mL. The age median patients who were HCV-negative was 56 year, III-IV stage was at 59 pts, I-II at 49 pts. Normal level ALT and AST was at 102 pts. All pts were treated by CHOP. Among 108 pts without markers HCV infection increased serum ALT and AST only at 15 pts. The median was 2.3 norms. HCV RNA it was not defined at all this 15 pts. The treatment has not been stopped at any pts thanks to hepatic toxicity. CR has been reached 56 with DLBCL. Median follow up was 28 months. Serum level HCV RNA has increased at 33 of 41 pts. At all 25 pts before therapy the median serum level HCV RNA. Level of HCV RNA was from 9x10^6 to 8.8x10^6/mL a median has made 6.7x10^6/mL. HCV RNA levels significantly increased during immunotherapy 21 from 41 pts simultaneously with increased serum level HCV RNA increased level ALT and AST. Level ALT was from 4 to 80 norms, median-11 norms. Level AST was from 2 to 90 norms. The reason of stop treatment at 15 pts was hepatic toxicity. Complete remission (CR) in pts with HCV infection has been reached 12 DLBCL. Median follow up CR was 12 months. In conclusion, our study showed a high incidence of severe hepatic toxicity in patients who were HCV-positive. Hepatic function should be limited in patients who are HCV-positive and receive immunotherapy. High-risk patients having chronic active hepatitis receiving cytokotoxic drugs, corticosteroids and rituximab should be closely monitored for serum transaminase, bilirubin and HCV RNA levels.

**DIFFUSE LARGE B-CELL LYMPHOMA WITH HEPATITIS C VIRUS INFECTION TREATED BY CHEMOTHERAPY WITH RITUXIMAB REGIMENS: PROGNOSIS AND TOXICITY**

V Sergey1, I Sorojakov1, A Kogrivina1, N Subhortova2, D Kosyural1, S Tumyna3, T Kondatyeva3, A Ettinger1, A Kolomiytsev3, A Zeinolova3, A Chekan1, L Timofeeva2

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Many epidemiologic studies have demonstrated an association between hepatitis C virus (HCV) infection and non-Hodgkin lymphoma, suggesting that HCV plays a role in the development of this malignancy. In a number of researches it is shown that level of various markers of a hepatitis C at patients from NHL can be taped at 30% of patients. In our research we studied function of a liver at patients with NHL carrying out immunohistochemistry with markers of a hepatitis C and without them. 41 patients (pts) with NHL have been included in research with markers HCV infection and 108 without it by which it has been spent immunohistochemistry. The age median patients who were HCV-positive was 47 years. 30 patients HCV-positive have III-IV stages of disease and II stage. Normal level ALT and AST prior to the beginning of treatment was 2.3x10^7/mL. The age median patients who were HCV-negative was 56 year, III-IV stage was at 59 pts, I-II at 49 pts. Normal level ALT and AST was at 102 pts. All pts were treated by CHOP. Among 108 pts without markers HCV infection increased serum ALT and AST only at 15 pts. The median was 2.3 norms. HCV RNA it was not defined at all this 15 pts. The treatment has not been stopped at any pts thanks to hepatic toxicity. CR has been reached 56 with DLBCL. Median follow up was 28 months. Serum level HCV RNA has increased at 33 of 41 pts. At all 25 pts before therapy the median serum level HCV RNA. Level of HCV RNA was from 9x10^6 to 8.8x10^6/mL a median has made 6.7x10^6/mL. HCV RNA levels significantly increased during immunotherapy 21 from 41 pts simultaneously with increased serum level HCV RNA increased level ALT and AST. Level ALT was from 4 to 80 norms, median-11 norms. Level AST was from 2 to 90 norms. The reason of stop treatment at 15 pts was hepatic toxicity. Complete remission (CR) in pts with HCV infection has been reached 12 DLBCL. Median follow up CR was 12 months. In conclusion, our study showed a high incidence of severe hepatic toxicity in patients who were HCV-positive. Hepatic function should be limited in patients who are HCV-positive and receive immunotherapy. High-risk patients having chronic active hepatitis receiving cytokotoxic drugs, corticosteroids and rituximab should be closely monitored for serum transaminase, bilirubin and HCV RNA levels.
Results. Of the nine patients, 55.6% were female with a median age of 63 years (range: 30-80). FLIPI was calculated: 0-1:4 (37%); II-IV: 24 (63%). Rescue treatments: chemotherapy with rituximab in group A and cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in group B. In patients treated in the rituximab group, the rate of CNS relapse was 7.9 months (range: 4-38.4 months). Maintenance with rituximab consisted of an infusion of 375mg/m2 every three months for 2 years in 9 patients and 2 courses of 4-times-weekly doses of rituximab, 3 and 9 months after completion of salvage therapy in 11 patients. 19 patients (47.5%) died after salvage therapy and 14 (52.5%) died. 20% of patients had infectious episodes and another 30% grade 3 or 4 neutropenia. There was no significant difference in age or FLIPI between both groups. Five patients received maintenance rituximab in second or later relapse so they are excluded for the efficacy analysis. The probability of salvage therapy failure was lower in the rituximab maintenance group: TTF median has not been reached in group A (60% without failure at 4.75 years) and it was 8.9 years in group B (p: 0.06). In the multivariate analysis only the FLIPI was significant (p:0.016) and almost significant the rituximab maintenance. The median of SV was 6.6 years in group A and 4 years in group B (p: 0.11). In the multivariate analysis FLIPI was the only significant variable (p:0.02). We did not observe significant differences in neuropenia between both groups, but infectious episodes were more frequent in A group (p:0.68). Summary/Conclusions. Our observations are consistent with the effectiveness described in clinical trials of rituximab as a consolidation of the chemotherapy response in first relapse of FL. The limited number of patients may justify the results did not reach statistical significance. The neuropenia in both groups and the infectious episodes were more frequent in the rituximab group. A favorable FLIPI was associated with less likelihood of treatment failure and a longer SV.

1449 CENTRAL NERVOUS SYSTEM INVOLVEMENT IN PREVIOUSLY TREATED DIFFUSE LARGE B-CELL LYMPHOMA - A SINGLE CENTER EXPERIENCE
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Background. The occurrence of central nervous system (CNS) involvement in patients with non-Hodgkin’s lymphoma, including diffuse large-B-cell lymphoma (DLBCL), is an uncommon (1.6% to 5%), but almost always fatal event. Available studies report a latency time from initial diagnosis to CNS relapse varying from 5.4 to 8.5 months. Prognosis is very poor with a median time from CNS involvement to death of 2 to 4 months. IPI, LDH and number of extranodal sites seem to be the more relevant risk factors in predicting the risk of secondary CNS involvement. Aims. To evaluate the patients with DLBCL treated in our center who presented with CNS relapse. Methods. Between October 2002 and January 2011, 20 patients were treated with R-CHOP (rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone) for DLBCL, presenting with CNS relapse. Data regarding diagnosis, staging, relapse clinical presentation and outcome was collected. Results. Of the nine patients, 56.6% were female with a median age of 66 years old (range: 53-80). At diagnosis, all patients were stage II or IV with IPI equal or superior to 3 and with elevated LDH (median=832; range: 220-1694; reference values: 67-190 U/L). Extraneural disease was present in 55.6% of patients; bone marrow was involved at diagnosis in 44.4%. Patients were treated in first-line with R-CHOP and 2 of them had intra-thecal prophylaxis with metothrexate. Six patients achieved complete remission (CR) at first-line (two of these had systemic relapse with achievement of a second CR) and two patients had resistant disease. Of the six patients in CR, five patients had an isolated CNS relapse. Both patients with resistant disease presented with CNS involvement while undergoing second-line therapy and one patient developed CNS disease before response to first-line therapy was evaluated. Median time from diagnosis to CNS relapse was 7.9 months (range: 4-38.4 months). Presenting symptoms of CNS involvement were paresis of lower limbs (n=5), visual impairment or diplopia (n=5), dysarthria (n=2), dysphagia (n=1) and palpebral proptosis (n=1). Work-up revealed parenchymal involvement (n=5), involvement of menoral (n=1) and optic (n=1) nerves; immunohistochemistry of cerebral spinal fluid (CSF) at relapse is positive in seven out of eight patients tested: five expressed CD20 and two were CD20 negative. Two thirds of the patients had died at the time of our study. Median survival after CNS involvement was 3.8 months (range: 0.7-39.2 months). Median overall survival of this group was 12.2 months (range: 5-72.4 months). Of the surviving patients only one is currently in CR after systemic and intra-thecal chemotherapy and autologous stem cell transplant. Conclusions. CNS relapse of DLBCL is an aggressive disease with a fatal outcome in most cases. Although all patients were advanced-stage, only two met criteria for intra-thecal prophylaxis and yet, it failed to prevent relapse. Recent literature questions the role of the this form of prophylaxis after the introduction of Rituximab. It is necessary to find more accurate risk factors in predicting the risk for CNS secondary involvement and develop more effective forms to prevent it.
3% of Fetal Bovine Serum at 37 °C and 5% CO2. Bone marrow non-adherent cells were removed after 24 h, and culture medium was refreshed twice a week thereafter. Cells grew adherent and with a fibroblastic morphology. We observed by flow cytometry that almost all cells expressed CD10, CD29, CD73 and α-SM-actin, but lacked CD45. Other antigens such as CD54, CD106, CD21, and STRO-1 were also detected. We used the collagen gel assay to determine MSC contractility. IL-2, IFN-γ, TNFα, or IL-6 (all them Th1 cytokines) increased MSC contractility, whereas that IL-10 (a Th2 cytokine) or cytokalasin (an inhibitor of actin polymerization) decreased MSC contractility and induced cell relaxation. By confocal microscopy, we observed an increase in the number of α-SM-actin+ stress fibers in the MSC treated with IL-2, IFN-γ, and TNFα, whereas IL-10 decreased that number. Our results show that the MSC contractility is modulated by cytokines that regulate the incorporation of α-SM-actin into the stress fibers.

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ANALYSIS OF CMV SPECIFIC IMMUNITY RECONSTITUTION IN PATIENTS FOLLOWING ALLOGENIC STEM CELL TRANSPLANTATION BY TETRAMER TECHNOLOGY

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CMV specific memory CD8+ T lymphocytes (CMV CD8+ T ly.) play crucial role in the regulation of CMV infection. The tetramer technique is an important tool for detection and quantification of CMV specific T cell response. The aim of the present study was to investigate the role of donor CMV serological status on CMV CD8+ T ly. recovery following allogenic stem cell transplantation and the influence of graft versus host disease (GvHD) treatment and anti-thymocyte globulin (ATG) application in patients more than 100 days after transplantation. In cases with seropositive donors, CMV CD8+ T ly. reconstitution was observed approximately at day 20 following transplantation. In 5 (45%) patients with seropositive donor and without GvHD we found high values of CMV CD8+ T ly.: mean 26 b/µl (min. 16 b/µl, max 44 b/µl). 80% received ATG. In 6 (55%) patients without GvHD and CMV seronegative donors we found low numbers of CMV CD8+ T ly.: mean 1,6 b/µl (min. 0 b/µl, max 6 b/µl). 83% received ATG. 20 (65%) patients with both seropositive and seronegative donors were diagnosed and treated for GvHD. In 14 (70%) cases with low CMV CD8+ T ly. levels (mean 0, 9 b/µl, min. 0 b/µl, max 4 b/µl) 57% received ATG. In 6 (30%) cases with high CMV CD8+ T ly. levels (mean 11 b/µl, min. 7 b/µl, max 24 b/µl) 80% received ATG. CMV infection was not diagnosed in any of the above mentioned cases. Our results are in agreement with the hypothesis that donor serological status play crucial role in CMV specific immunity reconstitution. Our observations demonstrate significant negative influence of GvHD treatment on CMV specific immunity, while no effect of ATG application was proven.

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ACUTE GVHD IS A STRONG PREDICTOR OF FULL DONOR CD3+ T CELLS CHIMERISM (TCC) AFTER REDUCED INTENSITY CONDITIONING (RIC) FOR ALLOGENIC STEM CELL TRANSPLANTATION (ALLO-SCT)

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The monitoring of chimerism is a standard procedure to assess engraftment and achievement of full donor lymphoid chimerism after RIC. Post graft donor lymphocyte infusions are often determined on this study. However there is no consensus on when and how often to perform post-transplant chimerism. We retrospectively analysed our experience about the impact of acute GVHD in the prediction of allograft chimerism. All patients with hematologic malignancies excluding myelofibrosis, transplanted between 2001 and 2010 after Fludarabine-Busulfan-ATG RIC from a HLA identical donor and with T cells chimerism (TCC) determination between day 30 and 120 were included. 115 patients fulfilled all criteria. Alto-SCT was performed from familial donor in 92 patients (80%) and from MUD in 23 patients (20%). The conditioning regimen consisted of Fludarabine 90 to 180 mg/m2, Busulfan 8 mg/kg orally or 6.4 mg/kg iv and ATG 2.5 or 5 mg/kg. TCC was serially assessed at 30, 60 and 90 days after allo-SCT. Recipient peripheral blood lymphocytes were positively sorted by annex 5% of anti-CD4 and CD8 immunomagnetic beads (Dynal, Compiegne, France). T-cell purity was controlled by flow cytometry and was always > or =95%. Genomic DNA was amplified using fluorescent PCR primers for polymorphic variable number tandem repeats (VNTR) or short tandem repeats (STR). Mixed T-cell chimerism was defined as between 5 and 94% recipient cells, and full chimerism was defined as the presence of more than 95% donor cells. Full TCC was achieved in 94 patients (82%) at a median of 77 (30-120) days post transplant. Fifty eight patients (50.4%) developed acute GVHD. The cumulative incidence of Grade 2-4 GVHD in our population is 32% (95% CI 23-41). Overall the results showed that each of the 37 patients developing grade >= 2 AGVHD had a Full TCC prior day 120. On the other hand, all mixed chimerism were documented in patients not presenting Grade>=2 AGVHD (21 of the 78 patients (27%) without grade >= 2 AGVHD) (p=0.002). No other parameter (ATG dose, Donor type..) achieved this level of individual

Figure 1. Monitoring of DNA markers after alo_SCT
prognostic. These results, in a very homogenous population, raise the question concerning the utility of routine chimerism surveillance in patients presenting an acute GvHD following RIC Allo-SCT and that can imply a not negligible saving in terms of economic and human resources.

**Figure 1:** Cumulative incidence of acute GvHD. (Kaplan Meier).

**MONOCYTE SUBPOPULATIONS AND THEIR DIFFERENTIATION PATTERNS DURING POST TRANSPLANT ADVERSE EVENTS**

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Background. Transplant-related adverse events are a major cause of morbidity and mortality in pediatric patients after high-dose chemotherapy and hematopoietic stem cell transplantation (HSCT). T-cell depletion of the graft, post-transplant immune deficiency, extensive immunosuppression, and neutropenia are known risk factors for bacterial and viral infections. Most of the data about monocytes during infection are known from murine models. Less is known, however, about the role played by monocytes in defense against infections post-HSCT. Monocytes consist of several subpopulations, which can be classified according to cell-size, granularity and different expression levels of CD16/CD14. Blood monocyte subpopulations have been defined in two major types CD14++/CD16- (M1), and CD14+/CD16++ (M3) and the intergradation between these subpopulations the CD14++/CD16+ (M2). We conducted a pilot study and analyzed the monocytes subpopulation during post transplant period and transplant related adverse events. Patient and Methods. The patient group consisted of 80 pediatric patients and young adults (7 autologous, 12 allogeneic and 11 allogeneic haploidentical transplanted) with hematological malignancies and immunodeficiency disorders. The median age was 9.5 years (range 0.5-38 years). The period of analysis was from the day before start of conditioning until 365 days after HSCT. Normal values of monocyte subpopulations were analyzed from a control group of healthy children and young adults (n=20). Surface marker expression (CD14, CD16, CD64, HLA-DR) of monocytes was determined by four-color flowcytometric analysis. Results. The median analysis period in the patient group was 228 days (range 43-379 days). Post transplant a significant (p<0.001) increase of monocytes was observed at time of “leukocyte -take”, defined as the point in time after HSCT which leukocytes first reached levels above 1000/µl. We analyzed a subpopulation of monocytes, (M4), which has been up to this point difficult to distinguish from natural killer cells and shows a surface expression of CD14/CD16++. Looking at both the healthy population as well as pediatric patients and young adults after HSCT. The largest percentage of monocyte subpopulation in healthy subjects was M1 (85.3%) followed by M3 (7.5%), M4 (4.2%) and finally M2 (3.0%). In contrast with healthy populations, between day +30 and day +100 after HSCT the proportional share of M1 (74.5%) presented as too low, while M2 (7.9%), M3 (11.3%) and M4 (6.3%) were rather high. While M4 already showed a significant decrease (p<0.05) five days prior to bacterial infection, a significant increase in M4 (p<0.01) was observed at the moment of viral infection. During acute graft-versus-host disease (GvHD) grade III and IV a significant decrease was observed in M1 (p<0.01), but a significant increase in M4 (p<0.05) and M5 (p<0.05). Summary. Altered patterns of monocyte subpopulations were observed during immune reconstitution and during post transplant adverse events. The role of the individual monocyte subpopulations remains to be elucidated during these conditions.

**LASER TRANSMYOCARDIAL REVASCULARIZATION (LTMR) WITH AUTOLOGOUS BONE MARROW CELLS (ABMC): ONE STEP METHOD FOR HARVESTING AND PROCESSING. EXPERIENCE IN 21 PATIENTS**

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Background: Recent studies suggest that Laser Transmyocardial Revascularization (LTMR) with Autologous Bone Marrow Cells (ABMC) could improve the results as cell therapy in diffuse coronary disease (DCD) respect to laser alone. We evaluated a point of care device which utilizes density gradient centrifugation to concentrate bone marrow mononuclear cells for transmyocardial laser injection. Patients and Methods: 21 patients with DCD and medically refractory class III/IV angina (mean age 66 years old), has been included in the study. At the time of surgery, 120 cc of autologous bone marrow were aspirated from the posterior iliac crest and anticoagulated with citrate. Using a density gradient centrifugal system (HARVEST®, Palex, USA), bone marrow harvested was separated into its components (Figure), which included 20 cc of concentrated mononuclear cells inclusive of the buffy coat which was immediately available for direct transmural myocardial laser channels injection. Cell counts and flow cytometry were used to determine the total number of mononuclear cells in addition to specific somatic stem cell populations such as CD34+ and CD133+ cells. Results: Time for bone marrow aspiration and concentration averaged 30 minutes. The complete processing was performed closed to the surgery room, in sterile ambient. There were no complications related to the bone marrow aspiration. Average cell counts pre and post concentration were significant (p<0.001) increased (Table). No correlation between cell counts and patient demographics was detected. All patients received laser therapy with cell support without complications. 19 patients were evaluable for results. Conclusions and comments: These interim data shows that density gradient centrifugation with the HARVEST® device allows fast and efficient point of care concentration of ABMMC for cell-based therapies. Efficacy results regarding cardiovascular applications and clinical endpoints will be presented.
Background: Patients with advanced hematologic malignancies after allogeneic hematopoietic stem cell transplantation (HCT) have a poor prognosis because of a high rate of relapse and transplant-related mortality. Donor lymphocyte infusion (DLI) after allogeneic HCT exhibits definite anti-leukemia effects in this group of patients. Our primary data showed the feasibility of modified prophylactic DLI in HLA-mismatched HCT without in vitro T-cell depletion, but there have been no comparative clinical studies to confirm its efficacy. The purpose of this non-randomized, single-center study was to comparatively analyze transplantation outcomes in a consecutive series of advanced-stage acute leukemia patients who either received prophylactic DLI or did not receive prophylactic DLI after HSCT from HLA-mismatched family donors during the same period at our institute. Methods The study was approved by the Institutional Review Board of the Peking University Institute of Hematology. All included patients were informed and signed an informed consent form. Consecutive patients with advanced-stage acute leukemia (patients in CR5 or beyond, non-remission, n = 75) receiving HSCT from HLA-mismatched family donors during the same period (between January 2003 and September 2010) with (n = 48) or without (n = 27) prophylactic donor lymphocyte infusion (DLI) were enrolled. Results Forty-eight patients received the prophylactic DLI at a median of 48 (28-330) days after HSCT. The cumulative incidences of overall grades II-IV acute GVHD were 48% for patients receiving prophylactic DLI and 53% for those not receiving prophylactic DLI, respectively (P = .85) with a relative risk (RR) of 0.91 (1.23-0.61) (P = .51). The cumulative incidences of overall chronic GVHD (including GVHD occurring before and after DLI) were 51% for patients receiving prophylactic DLI and 39% for patients not receiving prophylactic DLI, respectively (P = .42) with a relative risk (RR) of 1.99 (0.68-5.84) (P = .21). The 2-year cumulative incidence of relapse was significantly lower in patients receiving prophylactic DLI (34%) than in patients not receiving prophylactic DLI (54%) (P = .013). Summary/conclusions The current study showed that a lower relapse rate, a similar NRM, and a higher survival probability was achieved with patients receiving prophylactic DLI than with patients not receiving prophylactic DLI. The results suggest that prophylaxis of relapse with modified donor lymphocyte infusion can significantly increase survival for advanced-stage acute leukemia patients even after HLA-mismatched T-cell-replete HCT.

Figure 1. Overall Survival for patients receiving DLI or not.

The three-year probability of overall survival was higher in patients receiving prophylactic DLI (26%) than in patients not receiving prophylactic DLI (12%) (P = .415). Summary/conclusions The current study showed that a lower relapse rate, a similar NRM, and a higher survival probability was achieved with patients receiving prophylactic DLI than with patients not receiving prophylactic DLI. The results suggest that prophylaxis of relapse with modified donor lymphocyte infusion can significantly increase survival for advanced-stage acute leukemia patients even after HLA-mismatched T-cell-replete HCT.

GVHD PROPHYLAXIS WITH HIGH DOSE CYCLOPHOSPHAMIDE AFTER HLA-MATCHED OR HAPLOIDENTICAL ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background: recently, it was reported that after T-cell replete bone marrow transplant (BMT) from HLA-matched1 or haploidentical (haplo) related donor2, post-transplant cyclophosphamide (Cy), respectively given alone or with Tacrolimus (FK-506) plus Mycophenolate Mofetil (MMF) for GVHD prophylaxis, was associated with low incidence of graft rejection and acute and chronic GVHD (aGVHD, cGVHD) in patients (pts) with advanced myeloid malignancies (AMM). Methods: in our centre, 8 pts with AMM (1 CML in blast crisis, 1 CML in accelerated phase, 3 AML not in CR in CR2 relapsed after autologous BMT) underwent unmanipulated BMT from HLA-matched (2) and haplo (6) donors (GVHD prophylaxis was performed with Cy 50 mg/kg at days (d) +3 and +4, alone or with FK-506 and MMF in HLA-matched and haplo BMT, respectively. In the haplo BMT, the conditioning regimen (Cond) was Cy 14.5 mg/kg at d. -6 and -5, fludarabine 30 mg/m2 from d. -6 to -3 and TBI 200 cGy at d. -1 in 4 pts; in a patient Cy was substituted with thiopeta 5 mg/kg at d. -6 and -5. In HLA-matched BMT, Cy was administered fludarabine 120 mg/m2 and busulfan i.v. 12.8 mg/kg in 4 days. Results: the median time to reach ANC>500/microl was 18 d. (r. 13-26) in 8/8 of pts, and platelets>30000/microl was 29 d. (r.19-40) in 7/8 of pts. After a follow-up of 8 months (m.) (r. 2-23), 5 pts are alive in CR (haplo and HLA-matched BMT in 4 and 1 case, respectively). Two pts (25%) developed steroid-responsive grade 2 cutaneous aGVHD at d. +18 and +39. After FK-506 and MMF withdrawing, 2 pts undergone haplo BMT had delayed grade 2 intestinal GVHD, responding to standard therapy. Interestingly, Foxp3 levels were significantly low in pts developing aGVHD. None of our pts showed cGVHD. Viral infections were documented and cured in 66% of pts. A patient (12.5%) died for transplant-related complications at 2 m. while 3/5 (60%) relapsed and 3/5 (60%) died after 4.5 and 8.5 m. Both these pts had refractory AML at the transplant and received BM from haplo donors. Conclusions: post-transplant Cy is a safe and effective prophylaxis of aGVHD and cGVHD in HLA-matched or haplo allogeneic BMT. To reduce relapse rate, especially after haplo BMT in pts with active disease, we are proposing clofarabine and busulfan i.v. and planned in a multicenter study of GVHD prophylaxis with high-dose Cy after allogeneic BMT.

PLANTATION FOR MULTIPLE MYELOMA WITHOUT GROWTH FACTORS: outcomes are scanty. Aims: We analyzed and updated our experience on from peri-transplant infections. Conclusion: it seems that LGL expansion is strongly associated with chronic GVHD and with graft versus leukemia effect also in absence of full donor chimerism. We think that pts undergone allo-HSCT presenting LGL expansion need to be monitored carefully for systemic chronic GVHD. Further study in larger series of pts are needed to evaluate this complex mechanism.

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SPLENECTOMY AND HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR THALASSEMIA PATIENTS

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Background: beta thalassemia is a genetic disorder resulting in absent or reduced beta globin chain synthesis producing haemolytic anemia. Currently, the only cure for thalassemia is allogeneic bone marrow transplantation, which corrects the genetic defect in the hematopoietic system but does not cure the underlying disease. The use of allo-HSCT, the origin of which must be immunologically acceptable. Patients with thalassemia can be categorized into 3 classes of risk for bone marrow transplantation (Pesaro classification), and the category of greatest risk is class 3. Aims: massive splenomegaly in patients with thalassemia is often a reflection of inadequate medical care and/or advanced disease and frequently is found in class 3 patients. It is also associated with increased blood transfusion requirement. Splenectomy is indicated if there is significant abdominal discomfort, splenic infarction, or synchronous hypersplenism. Presence of splenomegaly prior to an SCT raises the theoretical concern of the removal of infused stem cells, which could potentially have an adverse impact on engraftment. Given this, splenectomy performed prior a SCT could modify engraftment kinetic, which in turn could have an impact on graft tolerance and development of GVHD. Methods: our experience runs on fifteen patients affected by thalassemia that underwent splenectomy in the Department of Surgery of the Tor Vergata University General Hospital of Rome from May 2005 to April 2010. All patients were prepared for surgery by preoperative blood transfusion to achieve more than 9.5 g/dL of haemoglobin level and all received prior pharmacological immunization with vaccines for meningococcus, pneumococcus and haemophilus influenzae. Results: mean and median age were 11 y 30 (min 4 - max 26 yrs). Mean operative time for surgical procedure was 75 min (range 60 - 100 min). The average postoperative hospitalization time was 3.7 days (range 5 - 7). Minimum spleen weight was 495 g and maximum 2397 g. Only one patient at day +2 after surgery showed increased level of amylases and lipases, but he was given promptly i.v. sandostatine treatment and those above mentioned enzymes normalized in five days. Conclusions: in this retrospective analysis, we reported that splenectomy prior to an allogeneic SCT in patients with thalassemia is indicated in case of massive splenomegaly, a reduced peri-transplant transfusion requirement, and with not a significantly increased risk of death from peri-transplant infections.

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HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA WITHOUT GROWTH FACTORS: EXPERIENCE FROM A DEVELOPING COUNTRY (ORAN, ALGERIA)

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Background: The need for growth factors after autologous stem cell transplantation (ASCT) has been investigated recently. Data from developing countries are scanty. Aims: We analyzed and updated our experience on 32 consecutive patients with multiple myeloma (MM) treated with ASCT without receiving growth factors after transplant. Patients and methods: Among the 32 patients 11 was females and 21 was males. The median age was 55 years (range, 37-64 years). Before transplant, patients received chemotherapy using VAD (vincristine, adriamycin and dexamethasone, n=10) or bortezomib-dexamethasone (n=22). The median number of CD34+ cells was 8.6x10^6/kg (range, 1.05 - 8.62). High-dose melphalan (200 mg/m²) was used for conditioning and followed after 24 hours by reinfusion of autologous non-frozen hematopoietic stem cells, which had been stored for 24 hours at 4°C. All patients received prophylactic ciprofloxacin, fluconazole and acyclovir. Results: All evaluable patients had a full hematopoietic reconstitution. Median time to achieve neutrophils ≥500/μl was 12 days (range 10 - 17) and median time to achieve an unsupported platelet count ≥20 000/μl was 13 days (range, 11 - 28). All patients met the criteria of ES, 27% of patients developed acute GVHD 1-2°, 21% grade 3°, 7% grade 4°, and 5% of cases the major regimen related toxicity. The median follow-up was 7 months (range,1-20 months). Estimated overall survival and EFS at 20 months were 96.5±0.05% (s.e.) and 93%±0.05% (s.e.), respectively. Conclusion: We conclude that it is feasible and reasonable to perform ASCT for MM without administering growth factors and the procedure is easy to perform without requiring costly growth factors.
IMPACT OF PRIOR AUTOLOGOUS MOBILIZATION STATUS ON ENGRAFTMENT AND OUTCOME AFTER ALLOGENEIC STEM CELL TRANSPLANTATION FOR LYMPHOMA AND MYELOMA

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Aims: approximately 15% of patients who are candidates for autologous HSCT fail to collect an adequate amount of CD34+ cells (poor mobilizers). Reasons for poor mobilization include patient and treatment-related factors. Infra-clinical damages to the bone marrow (BM) micro-environment may play a role and affect the interactions of stem cells with stromal cells and the bone-blood barrier that contribute to efficient mobilization. If such a hypothesis stands true, then damages to the BM micro-environment should also affect the ability of donor stem cells to home and seed in the BM of recipients after allogeneic HSCT.

Methods: inclusion criteria were age > 18 years, diagnosis of lymphoproliferative disease or multiple myeloma, prior mobilization (successfully or not), HSCT from related or unrelated donor and RIC conditioning. Poor mobilization was defined as circulating CD34+ <20/µl on the first day of planned collection. PMN and PLT engraftment were determined for good and poor mobilizers, taking into account confounding factors such as age, type of donor and disease. Results: a total of 148 patients were included in this analysis, of which 125 were evaluable for CD34+ counts and engraftment (n=33 for poor-mobilizers and 92 for good mobilizers, see table 1). All but two patients engrafted. Overall, the median (range) day was 17 (0-47) for PMN >0.5 G/L, 8 (0-49) for PLT >200G/L and 12 (0-49) for PLT >500G/L. There was no significant difference in PMN or PLT engraftment between the two subsets. Nevertheless, NRM and overall mortality were higher in poor mobilizers (p=0.099 and p=0.01 respectively); relapse rate, acute and chronic GVHD were similar in both groups (p=0.81). Data were confirmed after adjustment for patient’s age, type of donor and disease. Conclusions: our data suggest that lymphoma and myeloma patients with a history of poor mobilization defined as CD34+ <20/µl do not have different outcomes in terms of PMN and PLT engraftment. Despite the absence of difference in terms of early hematopoietic recovery after allogeneic HSCT, NRM and mortality were significantly higher in poor mobilizers. Identifying the exact reasons for such different outcomes will require further investigation.
4 are alive with persistent disease. Conclusions. From our preliminary data in a small series of CLL patients, the first 18F-FDG PET-CT after transplant shows different metabolic findings that reflect the different pre-transplant status and seem to predict the patient outcome earlier than clinical evaluation by standard criteria. PET-CT performed during follow-up is useful to assess disease status and to early detect disease progression.

1466
ETANERCEPT FOR STEROID-REFRACTORY ACUTE GRAFT-VERSUS-HOST DISEASE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Treatment strategy for steroid-refractory acute GVHD after Allo-SCT needs to be standardized. We report our clinical experience of the effect of etanercept on steroid-refractory acute GVHD. A total of 18 patients who received allo-SCT and presented with steroid-refractory acute GVHD at Aju University Hospital were retrospectively studied. Twenty-five milligrams of etanercept were given s.c. twice weekly for four weeks. Clinical responses were evaluated with regard to the severity of acute GVHD. The median age of patients was 43.5 years. By using paired t-test, etanercept showed down grading effect of acute GVHD (p = 0.005) but no patient experienced complete remission. Eighty percent of grade II, 14% of grade III, and 57% of grade IV patients showed a partial response. Skin and gut GVHD were well controlled with etanercept whereas hepatic GVHD was not. Four patients died of fatal infection. There were no factors affecting the clinical outcome of etanercept. All the non-responders died and 56.6% of the responders survived (p = 0.0008). Etanercept can be effective in treating steroid-refractory acute GVHD after Allo-SCT, with tolerable side effects.

1467
VON WILLEBRAND FACTOR (VWF) LEVEL IN THROMBOTIC MICROANGIOPATHY (TMA) IN CHILDREN UNDERGOING ALLOGENEIC BONE MARROW TRANSPLANTATION: A SINGLE - CENTER EXPERIENCE

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Introduction: Thrombotic microangiopathy is a well-documented complication that may occur after hematopoietic stem cell transplantation (HSCT). The reported incidence of TMA after HSCT varies from 0.5% to 75%, is characterized by thrombocytopenia, microangiopathic hemolytic anemia, fever, neurologic and renal abnormalities resulting from formation of disseminated hyaline thrombi in the small vessels of the main organs due to generalized endothelial damage. VWF is a multimeric protein contained in endothelial cells, platelets and plasma that mediates platelet adhesion and aggregation at sites of vascular injury. Once released from the abnormally stimulated endothelial [by multiple factors: high-dose chemotherapy, immunosuppressants, graft-versus-host disease (GVHD), infection and irradiation] ultra-large multimers of VWF, present in the endothelium but not found in the plasma in physiological conditions, directly promote intravascular aggregation of the platelets and the consequent microvascular thrombosis. Materials and methods: We report a retrospective analysis of patients (15 months to 17 years old) with hematologic disease who underwent allogeneic HSCT between February 2007 and December 2009 at our institution. We diagnosed transplantation-associated TMA in 5 of 36 transplanted patients. Table 1 shows the characteristics of patients who developed TMA after HSCT. The diagnosis was evaluated on the basis of clinical and laboratory criteria: microangiopathic hemolysis with negative Coombs test, unexplained drop in hemoglobin level and platelet count (Plt), increased number of reticulocytes (Ret), evidence of red cell fragmentation on the blood smear, increased value of indirect bilirubin (double of the normal value), increased serum lactate dehydrogenase (LDH) activity (more than 500 U/l), renal dysfunction (serum creatinine = Cre > 1.5 mg/dl), neurologic dysfunction. In addition, all patients were monitored for VWF levels (VWF Antigen kit-ELISA). Results: The most relevant clinical and laboratory data concerning diagnosis, therapy and patient outcome with TMA after HSCT are listed in table 2. Five of 36 transplanted patients (15.8%) developed TMA for a median duration of 58 days (range, 21-90). In 3 of 5 patients TMA occurred within 100 days after HSCT. All patients had increase in fragmented red cells, thrombocytopenia, significant elevation in LDH. Moreover we found elevated FvW levels until the early signs of thrombotic microangiopathy, even in a case was the first parameter to be altered with a modest decrease in platelets. Of these 5 patients, 5 responded to administration of Df + PE and only one developed, during DF treatment, severe TTP and died from multiorgan failure. Conclusion TMA is a severe and relatively common complication after HSCT. The diagnosis in transplanted patients is difficult because its presentation overlaps with other post-HSCT complications. However is important to recognize it before TMA can have harmful effects. This retrospective analysis of transplanted patients in BMT Unit of Pediatric Center evaluate the impact of TMA and the relevance, even in our small series, of VWF levels as important parameter for the diagnosis and monitoring the evolution of the disease.

1468
DOUBLE UNRELATED CORD BLOOD TRANSPLANTATION FOR ADULTS WITH HIGH-RISK ACUTE LYMPHOBLASTIC LEUKEMIA IN KOREA

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Background. Unrelated cord blood (CB) has been shown to be a valuable alternative source of hematopoietic stem cells for transplantation in patients who lack a suitable related or unrelated donor. Graft cell dose is an important determinant of hematopoietic recovery and overall outcome following CB transplantation (CBT), and the limited cell dose of single CB unit has been a major barrier to its more widespread use. Strategies to overcome this barrier include the use of two partially HLA-matched CB units (double CBT). Aims: We report the results of unrelated double CBT for 14 adults with high-risk acute lymphoblastic leukemia (ALL) between 2005 and 2010. Methods: The median patient age was 31 years (range, 16-52 years). All patients had one or more high-risk features, including 7 Philadelphia chromosome-positive ALL. Eleven patients (78.6%) were transplanted in CR1; 1 (7.1%) in CR2; and 2 (14.3%) in refractory/relapsed status. All patients received myeloablative preparation consisting of total body irradiation (12 Gy), fludarabine (150 mg/m²), and cytarabine (9 g/m²). Graft-versus-host disease (GVHD) prophylaxis consisted of tacrolimus and mycophenolate mofetil. CB unit was selected according to the number of total nucleated cells (TNC) per recipient’s weight and HLA compatibility (HLA-A and -B by serotyping and HLA-DRB1 high-resolution genotyping). No patients had a suitably matched related or unrelated donor available at the time of transplantation. All patients and donors provided written informed consent, and the treatment protocol was approved by the institutional review board of The Catholic University of Korea. Results: The median cell doses infused were 3.60x10^7 TNC/kg (range, 2.62-7.88), 1.80x10^5 CD34/kg (range, 0.65-6.85), and 8.04x10^7 CD8/kg (range, 6.24-13.49). All patients achieved a successful engraftment with full donor chimerism. Neutrophil recovery occurred at a median of 25 days (range, 17-109 days), and platelet recovery occurred at a median of 39 days (range, 20-185 days) after CBT. Acute GVHD was observed in 9 patients (64.3%); 7 grade II, 2 grade III). Four (36.4%) of the 11 evaluable patients had chronic GVHD (2 mild, 2 moderate). With a median follow-up duration of 18 months (range, 5-58 months) for surviving patients after CBT, 10 patients are alive with a leukemia-free status, while the other 4 patients have died

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of relapse (n=3) or infection (n=1). The cumulative incidence of relapse and the probability of disease-free survival at 2 years were 23.8% and 76.2%, respectively. Summary/Conclusions. Our data suggest that adult high-risk ALL patients without suitable related or unrelated donors should be considered as candidates for double CBT and provide the rationale for a larger clinical study in Korea.

1469 SINUSITIS IN PATIENTS UNDERGOING ALLOGENIC BONE MARROW TRANSPLANTATION

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Background. Sinusitis is one of the most common infections in general population, but data on its incidence and impact on the overall survival in hematologic patients undergoing allogeneic bone marrow transplantation is scarce. Aims. Analysis of the frequency of sinusitis, its fatality and potential predisposing risk factors in allogeneic bone marrow transplant recipients. Methods. The records of 113 patients with different hematological diagnoses with the exception of chronic myelogenous leukemia who underwent altogether 128 allogeneic bone marrow transplantations, were retrospectively reviewed for the occurrence of sinusitis, its complications, survival and the transplant data. Results. Twenty nine patients (26.5%) developed sinusitis between day -1 and +2044 (269±376) post transplantation, with 43% of them within the initial 100 days. They were treated with antibiotics and only 2 patients required surgical intervention and one extensive surgery. The only factor which predisposed to sinusitis in the analyzed group was slower neutrophils recovery (19 vs 16.6 days; p=0.05).

The type of donor (related vs unrelated, matched vs mismatched), source of stem cells (peripheral blood vs bone marrow), number of transplanted CD34+ cells/kg and CR status had no impact on the occurrence of sinusitis. Sinusitis did not deleteriously affect overall survival of transplant patients (p=0.08). Conclusions Sinusitis is a frequent infection in recipients of allogeneic hematopoietic stem cells, occurring most frequently during the first 100 days post transplantation. It can successfully be managed with antibiotics in the majority of them and has no impact on the overall survival.

1470 THE OUTCOME OF HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PRE AND POST MODERN THERAPEUTIC INTERVENTIONS

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Background. We report the outcomes of high dose chemotherapy (HDT) and autologous stem cell transplantation (ASCT) in the first 100 cases treated in a single institute over 13 years. The two main groups of patients were those with Myeloma and Non-Hodgkin lymphoma. We assessed the outcomes of HDT and ASCT pre- and post-introduction of modern therapy in Myeloma and anti-CD20 in CD20 positive lymphomas. We also analysed the prognostic indicators at presentation in each disease group and compared our results to other centres. Aims This single-centre study is aimed at assessing the outcome of HDT and ASCT in our institute and analysing the results in relation to:- Prognostic indicators, previous treatments received and outcomes for Multiple Myeloma (MM), Diffuse Large B-cell Lymphoma (DLBCL) and Hodgkin Lymphoma (HD). - Outcome in patients with DLBCL following ASCT in relation to previous exposure to anti-CD20. - Outcome of ASCT in MM pre- and post-introduction of Thalidomide. - Compare the outcome of different disease groups in our institute to those already reported by others and identify which group of patients benefit the most from ASCT.

Methods We analysed data from all patients treated with HDT and ASCT since the start of the service in 1997. The details of the patients, their disease and outcome at 100 days for the period including December 2009 have already been reported to the British Society of Blood and Marrow Transplantations (BSBMT). Results The median age of all patients that underwent ASCT was 53 (range 23-66). Overall survival (OS) at 1 year was 89.5% and progression-free survival (PFS) at 1 and 5 years were 85.7% and 57.4% respectively. Mortality rates at 3 months, 1 year and 5 years were 2.2%, 10.5% and 34.4% respectively. Two patients died within 100 days of transplant, both with NHL. Log-rank analysis demonstrated that those with HD had the best survival following ASCT compared to other groups (p-value 0.059). The main disease groups and the number of patients in each group analysed were: Myeloma (n=39), Hodgkin lymphoma (n=14) and Non-Hodgkin lymphoma (n=40). There was no significant difference in PFS or OS following ASCT for DLBCL between the 14 patients that received anti-CD20 as part of their initial therapy and the 11 patients that were anti-CD20 naive. Treatment of Myeloma with Thalidomide protocoled with Melphalan was not associated with survival benefit over ASCT. OS post ASCT. ISS and IPi scores at diagnosis were not significant in predicting outcome after ASCT in Myeloma and DLBCL (p-values 0.101, 0.081 respectively). Summary/Conclusions. The outcome of HDT and ASCT in HD was better than any other disease group, as was expected. Overall outcome for patients was better than the average outcome reported by BSBMT and EMMIT. ASCT was not associated with survival benefit following HDT and ASCT in patients treated after the introduction of anti-CD20 for CD20 positive lymphomas or the use of Thalidomide as first line treatment in Myeloma. However, the small numbers of patients in each disease group makes it difficult to draw firm conclusions.
Stepwise multiple regression analysis showed WBC count to be the best predictor of CE ($R^2=0.096$). Conclusions: Donors with haematological disease, older age and higher BMI were more likely to need a CVL for CD34+ cell collection compared to healthy donors. Procedures using CVL were shorter in duration time and obtained a higher PBV. However, the use of CVL has no effect on CE. Low WBC seemed to be the best predictor of CE. This fact suggests, to take into account, the PBV in regard to the pre-apheresis WBC in order to avoid the necessity of multiple apheresis sessions.

1472
THE CLEARANCE OF INFUSED HEMATOPOIETIC STEM CELL FROM THE BLOOD CIRCULATION
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Background. Homing is the essential step and is a rapid process in hematopoietic stem cell (HSC) transplantation. Aim and method: In the present study, using flow cytometry, we tried to determine the clearance time of the infused HSCs from the circulation following autologous HSC transplantation in 20 patients. Results. The median CD34+ cells were at the highest level at the first hour and reached below pre-infusion values at the first day after HSC infusion. By nonparametric analysis, positive correlation was determined between CD34+ cell levels at the first hour and neutrophil engraftment (r = 0.57, p = 0.01). Inverse correlation was found between CD34+ cell levels at the first hour and platelet engraftment. Conclusion. The highest level at the first hour and reached below pre-infusion values at the first day after HSC infusion. By nonparametric analysis, positive correlation was determined between CD34+ cell levels at the first hour and neutrophil engraftment (r = 0.57, p = 0.01). Inverse correlation was found between CD34+ cell levels at the first hour and platelet engraftment.

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1473
RISK FACTORS FOR MICROBIAL CONTAMINATION OF PERIPHERAL BLOOD STEM CELL PRODUCTS
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Background. In spite of the well known contamination rates and the microbial agents in stem cell products, the risk factors affecting microbial contamination have not been well described. Study Design and Methods: In a 12-year period, we retrospectively evaluated the culture results and risk factors after processing and thawing of a total of 510 peripheral blood stem cell (PBSC) products of 184 patients/donors. Results: Of 28 (5.7%) PBSC products in which microbiological contamination was detected after processing, growth was not detected in 13 (46.4%) products in the post-thawing period. Large volume leukapheresis (LVL) (odds ratio [OR]= 5.85, 95% confidence interval [95% CI] 1.52 - 22.49, p = 0.01) and the large number of the culture specimens that were sent (OR= 1.4, 95% CI 1.03 - 1.91, p = 0.03) were found to be risk factors for post-processing growth. The presence of post-processing microbial growth was found to be risk factor for post-thawing (OR= 20.22, 95% CI 6.67 - 125.15, p < 0.001) and post-transplant (OR= 3.25, 95% CI 1.24 - 8.50, p=0.01) microbial growth. Conclusion: Culture results should be monitored carefully in patients in whom post-processing growth risk increased when LVL was applied and when a large number of culture samples were sent. In transplantations performed with products having post-processing growth positivity, one must keep in mind the fact that growth of different pathogens may be seen at a high rate (80%) along with a markedly increased risk of culture positivity (OR=8.25).

1474
RISK FACTORS FOR ADVERSE EVENTS DURING COLLECTION OF PERIPHERAL BLOOD STEM CELL
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Background. Although collection of peripheral blood stem cells (PBSCs) by apheresis devices is a safe procedure, patients/donors-associated adverse events (AEs) and apheresis instrument related technical problems might be observed. Aim and Methods: We retrospectively reviewed of PBSCs collections following 528 mobilization cycles (413 patients and 75 related donors) over a 10-year period with the intent of identifying risk factors for AEs occurring in association with the procedures. Results. A total of 688 adverse events (13.1%) of which 142 (20%) related to the collection procedure. 191 (12.15%) of the AEs were classified as clinical AEs and 15 (9.5%) were classified as apheresis instrument-related AEs. The most common clinical AE was numbness of lips, tongue, or extremities (161 procedures, 10.2%) related to the infusion of acid citrate dextrose-A (ACD). Multivariate analysis revealed high amounts of ACD/weight (odds ratio [OR]=1.11, 95% confidence interval [95% CI] 1.02 - 1.21, p=0.009), high numbers of procedures (OR= 1.33, 95% CI 1.13 - 1.56, pc=0.001) and female gender (OR= 2.83, 95% CI 1.70 - 4.71, p<0.001) as being significantly associated with clinical AEs. Discussion. Gender was shown to be the most important risk factor for clinical AEs. Females who significantly increased risk of AEs would benefit from prophylactic calcium before and/or during PBSC collection.

1475
DOES SECOND ALLOGENEIC STEM CELL TRANSPLANTATION COULD BE TREATMENT OPTION FOR RELAPSES, GRAFT REJECTION OR ABSENCE OF ENGRAFTMENT AFTER FIRST ALLOGRAFT: SINGLE CENTER EXPERIENCE
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Introduction. Allogeneic stem cell transplantation (SCT) is only poten-
tially curative treatment option for different hematological malignan-
cies. Despite efficacy, relapses are still possible and till now there is no
standard approach neither for relapses, nor for other SCT failures. Cur-
cent options for such cases are chemotherapy, donor lymphocyte infu-
sion or second SCT. Aim: We are reporting outcome of second SCT in
our center. Patients and method. From 1995 till 2010., 18 patients (pts)
underwent second SCT for treatment of relapse (18 pts), graft rejection (2 pts) or absence of engraftment (3 pts) after first SCT. Median age in
this cohort of pts was 22.5 (16-52) years. M/F ratio 11/7. Pts were suf-
fering from various diseases: 2 AML, 9 ALL, 4 CML, 1 MDS, 2 AA. Medi-
an follow up is 30,5 (range 2-180) months. Results. Disease relapse had
occured at a median of 18,7 months after first allo SCT (range 6-72).
Graft rejection was observed after one year from first SCT in both
cases with aplastic anaemia. Pts with acute and chronic leukemias had
received salvage chemotherapy (Flag-IDA) and afterwards despite of mar-
row findings, underwent second allogeneic SCT with reduced intensity
conditioning. Pts with aplastic anaemia were conditioned with
Cyclophosphamide and ATG. All pts had received stem cells from same
identical sibling donor. Peripheral blood was source of stem cells in 16
pts and bone marrow in 2 pts. Engraftment was observed in all pts with
median neutrophil recovery after 16 (11-23) days. Prevention of graft
versus host disease (GvHD) was modified according to specific situation
(complete absence of prophylaxis in the cases of leukemia relapses or
combination of Cyclosporin A with Metothrexate or MMF in the graft
rejection or graft failure). Overall survival (OS) of all our pts is 57,5%
with median follow up 60 (2-180) months. Conclusion: Our modest
results have showed benefit of second SCT as treatment option for
selected cohort of pts who have failed after first allografting. Further
investigation on larger, homogenuos groups of pts is need.

1476
HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOIDE LEUKEMIA IN 77 PATIENTS UNDER 18 YEARS OLD
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Background. The prognostic of acute myeloid leukemia (AML) in chil-
dren has significantly improved over this last two decades drastically
with the help of the intensive chemotherapy. Several randomised trials have
shown the superiority of HSCT in children with high risk AML in
first complete remission (CR). This is a single centre retrospective study
involving in 77 children receiving HSCT for HLA identical sibling donors
for AML. Material and methods. From September 1998 to April 2010, 77
patients (pts) under 18 years old received an HSCT for AML (62 pts
were in 1st CR, 8 pts in 2nd CR and 4 pts were refractory). The cytolog-
ic types of AML (FAB: M1:16 pts, M2:27 pts, M3:3 pts, M4:15 pts, M5:4
pts, M6:14 pts) were classified as clinical AEs and 15 (9.5%) were classified as apheresis instrument-related AEs. The most common clinical AE was numbness of lips, tongue, or extremities (161 procedures, 10.2%) related to the infusion of acid citrate dextrose-A (ACD). Multivariate analysis revealed high amounts of ACD/weight (odds ratio [OR]=1.11, 95% confidence interval [95% CI] 1.02 - 1.21, p=0.009), high numbers of procedures (OR= 1.33, 95% CI 1.13 - 1.56, p=0.001) and female gender (OR= 2.83, 95% CI 1.70 - 4.71, p<0.001) as being significantly associated with clinical AEs. Discussion. Gender was shown to be the most important risk factor for clinical AEs. Females who significantly increased risk of AEs would benefit from prophylactic calcium before and/or during PBSC collection.

Discussion

Discussion
R.M. Gorbacheva Memorial Institute of Children Hematology and L Zubarovskaya, B Afanasyev
EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER
Free Survival at 12 years are respectively 56,3% and 55%.
still alive, 45 pts (58,4%) are in remission. Overall Survival and Event
of 16 month s (2-36). TRM incidence is 16, 8%. At the 30 th of Novem-
8%). Only 5 pts (6,4%) have presented CMV infection at median time
27). Forty two pts (54, 5%) needed blood transfusion, 72 pts (93,5%)
median time to achieve neutrophils count > 500/mm3 is at day 14 (8-
4 pts with ALL and 4 pts with AML. The mean age was 14.5 years
acute leukemia who relapsed after allo-HSCT 8 pts (10%) had ER includ-
leukemia ER after allo-HSCT. Results: Among 79 patients (pts) with
cohort single-center study of children and young adults who had
ent treatment options.

1477
TREATMENT EFICACY AND PROGNOSIS IN PATIENTS WITH
EXTRAMEDULLARY RELAPSE OF ACUTE LEUKEMIA AFTER
ALLOGENIC HEMATOPOETIC STEM CELL TRANSPLANTATION
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Background. Leukemia relapse still remains a problem in patients after
allogeneic hematopoietic stem cell transplantation (allo-HSCT). A lot of
relapses occurred in extramedullary sites with or without bone marrow
involvement. Extramedullary relapse (ER) seems to be less sensitive to
chemotherapy and immunotherapy, and there is no standard treatment of
pts with ER after allo-HSCT. Aims: To study frequency and clinical fea-
tures of leukemia ER after allo-HSCT and to evaluate efficacy of differ-
ent treatment options (BU, CY, ARA-C, ATG) performed 10-year-ret.
Method: A single-center study of children and young adults who had
leukemia ER after allo-HSCT. Results: Among 79 patients (pts) with
acute leukemia who relapsed after allo-HSCT 8 pts (10%) had ER includ-
ing 4 pts with ALL and 4 pts with AML. The mean age was 14.5 years
(range, 1-21). At the moment of allo-HSCT 5 pts had complete remis-
sion (CR), 2 pts had relapse, and 1 pt had refractory disease. Allo-HSCT
from matched unrelated and matched related donor was performed in
6 and 2 pts respectively. The conditioning regimen was myeloablative
and reduced-intensity in 5 and 3 patients respectively. The median time
between allo-HSCT and ER was 15 months (range, 3-26). Two pts had
isolated ER with full donor chimerism in BM. In other cases ER with
simultaneous bone marrow (BM) involvement (n=2), consecutive occur-
rence of ER and BM relapse (n=2) and BM relapse 3 and 10 months pri-
or to ER (n=2) were diagnosed. The sites of ER were head and neck, sal-
ivary glands, breast, paravertebral tissue, bones, skin, kidneys, testes,
ovaries, small pelvis. All pts received chemotherapy and immunoadop-
tive therapy with donor lymphocyte infusion (DLI). We retrospectively
analyzed 42 consecutive patients transplanted between Jan 2007 and
Oct 2010. Thirty-three patients were in first complete remission (CR1), 2 in second remission (CR2) and 7 patients in more advanced
stage with median age of 28 years (range, 17-55). The median follow-
up was 15 months (range, 1-48).

1478
THE USE OF ADJUSTED IDENTITY BODY WEIGHT FOR OVERWEIGHT
PATIENTS UNDERGOING HPC MOBILISATION FOR AUTOLOGOUS
TRANSPLANTATION
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Background. Generally, transplant protocols dictate the use of patients’
actual body weight (ABW) to calculate the required number of
CD34+ cells to be harvested for later hematopoietic progenitor cell
(HPC) transplantation. In recent years there has been a significant
increase in obesity rates in developed nations. In Australia, the 55-64 age
group had the highest combined rates of overweight and obesity. This
age group is overall the most common age patients proceed to autolo-
gous transplant for haematological malignancies. More overweight
patients requiring larger HPC collections to meet transplant target dos-
es per kg body weight, equates to a higher demand on apheresis, trans-
plant and HPC storage facilities. Aims. We have sought an improved
measure of body weight (termed transplant weight) to reach HPC tar-
gets for overweight and obese patients undergoing mobilisation and
subsequent autologous transplantation. Methods: In our centre for the
last 10 years, for patients weighing 25% more than their ideal body
weight (IBW) (defined as ‘overweight’), their adjusted ideal body weight
(AIBW) has been used as their transplant weight and is calculated as fol-
ows: AIBW = for males; 50 + 0.91 x (height (cm) - 152) and for females; 45
+ 0.9 x (height (cm) - 152). AIBW is then used for determination of minimum blood volume to be processed for HPC harvest of 2 x 106 CD34/kg as follows:Litres processed = 6.5 x transplant weight/CD34 per microl. peripheral blood. AIBW is also used for determination of CD34 cell dosage given at trans-
plant for overweight patients. Results: AIBW has been used at our insti-
tution for 65/155 (42%) of patients with haematological malignancies
who have had autologous HPC harvests. For patients > 25% above IBW,
amedian ABW was 90 kg (range 62-175 kg) whereas median AIBW was
69 kg (range 50-110 kg). Median volume of peripheral blood processed
to achieve minimum 2 x 106 CD34/kg using AIBW was 13.2 L (range 5-
35 L). For patients who then proceeded to transplant, 35/82 (45%) had
AIBW used to determine CD34 dosage/kg. All patients engrafted with
no significant difference between the groups. Median time to neutrophil
and platelet engraftment for overweight patients collected and trans-
planted using AIBW was 13 (range 10-24) and 15 (10-40) days respec-
tively. For normal weight patients collected and transplanted according
to their ABW, median time to neutrophil engraftment and platelet
engraftment was 12 (9-25) and 14 days (9-29) respectively. Conclusions:
By using an AIBW for patients more than 25% above their ideal
weight, we have reduced the volume of blood to be processed and
hence apheresis time required to achieve the minimum number of
CD34 cells per kg body weight. Further, this has reduced cryopreservation
storage space needed and dose of DMSO given at transplant. There has
been no adverse effect on engraftment times for these patients and we
consider this a safe and more efficient strategy for body weight estima-
tion for overweight patients undergoing autologous transplantation.1

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INTRAVENOUS BUSULFAN-CYCLOPHOSPHAMIDE AS PREPARATIVE
REGIMEN BEFORE ALLOGENEIC HEMATOPOIETIC STEM CELL
TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA
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Background. Intravenous busulfan-cyclophosphamide (IV BU/CY)
may improve the outcome of allogeneic hematopoietic stem cell trans-
plantation (allo-HSCT) by reducing the toxicity and TRM compare
with the oral BU/CY regimen. There are still limited reports of IV BU/CY
regimen in acute lymphoblastic leukemia (ALL). Aims. This study was to
evaluate the efficacy and toxicity of IV BU/CY in adult ALL patients
undergo allo-HSCT from HLA-matched donors. Methods. We retrospec-
tively analyzed 42 consecutive patients transplanted between Jan 2007
and Dec 2009. Twenty-nine patients were in first complete remission
(CR1), 2 in second remission (CR2) and 7 patients in more advanced
stage with median age of 28 years (range, 17-55). The median follow-
up was 15 months (range, 1-48).
Results. Overall, 13 patients died with 30-month OS at 56.0±10.6% (65.3±12.5% for CR1 vs. 54.4±15.3% for CR2 or beyond, p<0.001). Eleven patients relapsed 2 to 26 months after allo-HSCT with 30-month RR at 40.0±10.9% (32.0±12.7% for CR1 vs. 72.4±17.1% for CR2 or beyond, p=0.001). Overall, only 2 cases of clinical diagnosed VOD were documented (4.7%) and one died of severe VOD. Other conditioning associated toxicities were diffuse alveolar hemorrhage (DAH) in 1 and hemorrhagic cystitis in 8. A total of 4 patients died due to transplant related mortality (TRM) with 30-month TRM at 9.7±4.6%. Summary. This study demonstrated that the IV BU/CY can be considered as a feasible regimen for adult AL with low incidence of VOD and TRM.

**1480 PEGFILGRASTIM: SUCCESSFUL USE FOR PBSC MOBILIZATION IN ONE CENTER**

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**Background.** The use of G-CSF is most widespread method of peripheral blood stem cells (PBSC) mobilization during past decade. This cytokine may be applied alone or in combination with chemotherapy agents. When pegfilgrastim became available, great interest appeared to replace multiple injections of conventional form of G-CSF by single injection of pegylated form. We took an attempt to estimate mobilizing activity of pegfilgrastim given as single dose of 18 mg following chemotherapy (CT). The aims of our research were: 1) to reveal the ability of this regimen to produce a suitable peak of CD-34+ cells in adults with cancer diseases; 2) to prove the possibility to collect after this mobilization the sufficient number of PBSC for subsequent autologous transplantation; 3) to assess side events of such mobilization approach. Methods. Seventeen consecutive cancer patients (pts) were treated from April 2010 to February 2011. They received pegfilgrastim as a single dose of 18 mg in combination with CT to mobilize autologous PBSC. There were 5 female and 12 male, age was 24 - 55 y.o (median 40). Diagnoses were lymphoma (7), multiple myeloma (5), poor-risk Hogkin lymphoma (4), ALL (2), thymoma (1). Nine pts received Cyclophosphamide (CY) 6 g per b.s.m. followed by pegfilgrastim (single dose of 18 mg) shows high CD-34+ cell PB peaks, laid in restrict, well predictable time. Mobilized PBSC have high potency of hemopoietic reconstitution.

**Observations.** The high mobilizing activity of pegfilgrastim was found in our study. The PBSC collection rate was high in cases who received CT with CY and pegfilgrastim. It was 90% for pts treated with CY followed by pegfilgrastim, however, pts mobilized by other CT - on days 14 - 30 (median 19). Four of 15 pts were transplanted up today with fast and sustained engraftment. There were no considerable side events associated with mobilizing regimen. Conclusions. Our experience demonstrates that combination CT with pegfilgrastim for PBSC mobilization is safe and effective. CY 6 g per b.s.m. followed by pegfilgrastim (single dose of 18 mg) shows high CD-34+ cell PB peaks, laid in restrict, well predictable time. Mobilized PBSC have high potency of hemopoietic reconstitution.
estimated 30 days after MMSC infusion. The trial was approved by local ethic committee. Results. Among 4 patients with extensive chronic GvHD 2 patients didn’t respond, and both of them died from infections. One patient had a clinical improvement (alive for 15 months after HCT), and one patient had a partial response on the therapy (alive 24 months after HCT). Four patients with acute steroid-resistant GvHD grade II-IV were treated with MMSC. One patient didn’t respond (injection of MMSC +97 day after HCT) and died six months after HCT because of infections. One patient had clinical improvement (injection of MMSC +76 and +124 days) and one had complete response (injection of MMSC +82 day after HCT). They are alive 26 and 24 months without out immunosuppressive therapy and GvHD respectively. The fourth patient received 1 infusion of MMSC for acute steroid-resistant GvHD grades II after HCT (injection of MMSC +58 day) and complete response was achieved after this. Three months after HCT the patient relapsed and was treated with donor lymphocyte infusion, remission was achieved with GvHD grade IV. The patient received 3 infusions of MMSC and had partial response. The patient alive 11 month after HCT. The immunosuppressive therapy is continued. Conclusions. Good effect was observed in two cases among 4 patients with chronic extensive GvHD after MMSC infusion. In four among five cases with acute steroid-resistant GvHD injection of MMSC was effective. All of the patients who had response are alive, and patients who didn’t have response died. The best results are fired in cases when MMSC were infused directly after the diagnosis of acute steroid-resistant GvHD.

**Cardiac Complications after Bone Marrow Transplantation: A Report on a Series of 35 Cases in the Department of Bone Marrow Transplantation- Timisoara, Romania**

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Background. Cardiotoxicity is one of the non malignant complications after bone marrow transplantation and the principal clinical manifestations are: restrictive or dilated cardiomyopathy, arrhythmia, heart failure, pericarditis, hypertension, acute myocardial infarction. Aim: The purpose of this study was to estimate the frequency of the cardiac complications after BMT and of their impact on survival. Method: Cardiac complications related to bone marrow grafting were investigated in a group of 35 consecutive adults patients undergoing BMT (33 autologous, 2 allogeneic) in the transplant unit of Children’s Hospital- 3rd Clinic of Pediatrics Timisoara, Romania between 2008 and 2010. We followed the next parameters: heart rate, blood pressure, ECG, echocardiography, Holter ECG, Holter for blood pressure, chest x-ray. From 35 cases -5 patients have essential hypertension and 3 ischemic heart disease before transplant, without aggravation after transplant. Results. From 35 cases, 18 didn’t have cardiac complications and 17 with cardiac complications from 17 patients, 9 cases were Multiple Myeloma, 1AML, Hodgkin's Disease, 1 MDS (AREB II), 2 Non-Hodgkin’s Lymphoma. During the cells administration were no major cardiac complications. There were 2 cases of minimal pericarditis, 1 case of heart failure NYHA type II (EF<50%), 5 arrhythmias including 4 tachycardias and 1 ESV, 2 secondary hypertension, 7 diastolic dysfunction type I, 6 mitral regurgitation and 1 aortic regurgitation. After stem cell transplantation 9 cases developed dyslipidemia, considered one of the cardiovascular risk factors. The pregraft regimen was Melphalan, BEAM for autologous, Bu/Cy for allogeneic. Treatment before transplantation: VAD, Bortezomb+De-xamethasone for MM, R-ICE, R-CHOP, R-DHAP for NHL, ABVD, BEACOPP for HD, Cytosine-arabinoside + Anthracyclines for AML and MSD, mediastinal radiotherapy, were the factors basically responsible for the cardiac toxicity. Routine echocardiography confirmed the high incidence of subclinical cardiac abnormalities and their reversibility. The monitoring of the cardiac function is very important and the early treatment is essential; at these patients the cardiac treatment were with: beta-blockers=9 cases, ACE inhibitors=8, angiotensin-receptor blockers =2, statins=9, diuretics=1, nitrates=1, calcium channel blockers=1, antiplatelet agents=1. Conclusions. Currently we consider that the cardiac toxicity is one of the most important limiting factors for bone marrow transplantation. We suggest, therefore, that the transplantation should be done as early as possible. In our cohort didn’t appear major cardiovascular complications which required emergency cardiovascular intervention, only periodical follow-up. The major cardiotoxic events attributable to BMT are uncommon, comparable with another studies (in the literature occurring with a frequency of <1-2% after BMT).

**Ultrasound Detection of Delayed Focal and Diffuse Liver Diseases after Hematopoietic Stem Cell Transplantation in Children**

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Background. During Hematopoietic Stem Cell Transplantation (HSCT) the liver is the target of many type of injuries widely described in literature: toxicity related to the preparative regimen sinusoidal obstruction syndrome, hepatic acute graft-versus-host disease and acute hepatitis. In the setting of pediatric HSCT little is known about the role that HSCT may play in developing of late hepatic complications. At the state of art no guidelines exist regarding follow-up evaluation of liver status in pediatric patients after HSCT. AIMs. The aim of this study was to investigate the late effects of pediatric HSCT on the liver and to propose an adequate approach for monitoring liver status in post-transplanted children undergoing abdominal ultrasound (US). Methods. Evaluation of liver status using abdominal US was performed before and after HSCT in 51 children (22 girls, 29 boys) with a mean age of 9.4 years (range 0.5 - 21). All the patients underwent HSCT (15 autologous, 36 allogeneic) at our Institution. The follow-up evaluation with abdominal US have been performed every six months. RESULTS. At a median follow-up of 28.3 months (range 2 - 91) out of 51 patients evaluated, 5 (9.8%) presented focal liver disease at abdominal US: 3 patients developed focal nodular hyperplasia (FNH) and 2 hepatic hemangiomia. In 13 children we found the presence of diffuse liver disease (25.4%) with 10 cases of mild steatosis and 3 mild hepatomegaly. In 3 patients both steatosis and hepatomegaly have been observed. None of the patients had US hepatic disorders before HSCT. Characteristics of the patients who have developed hepatic disorders and of the HSCT procedure performed have been resumed in Table 1.


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Background. Graft versus host disease (GVHD) is a potential life threatening complication typically associated to autologous stem cell transplantation (AloSCt). Nonetheless, few reports in literature describe a GVHD-like syndrome occurring in patients undergoing an autolo-
THYROID DYSFUNCTION AFTER HSCT - A SINGLE CENTRE EXPERIENCE

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Background. Thyroid dysfunction after hematopoietic stem cell transplantation (HSCT) is recognized as a late complication. It has been established that both imaging and serological investigation are required in order to monitor the thyroid function of patients after HSCT. Aims: to present the experience of a single center SCT Unit in long term endocrinological follow up focusing on thyroid complications. Material and method: this is a single centre retrospective study of 146 consecutively admitted patients monitored for less than 5 years and thyroid dysfunction previously observed in 31 patients (21.2%). The most frequent problem was hyperthyroidism in the first 0-6 months after transplantation. All 7 (4.8%) developed hyperthyroidism in the first 0-6 months after transplantation. All 7 patients were started on oral non absorbable steroids, suggesting a key role of the gastrointestinal tract in the whole picture of the GVHD as it has been high-lighted in some animal models.

Conclusions. The incidence of thyroid dysfunction in our center is lower than that reported in the literature, probably because most patients were monitored for less than 5 years and thyroid dysfunction is a long-term complication in HSCT patients. Long-term survivors of HSCT are at increased risk for thyroid abnormalities. This knowledge should promote efforts for regular screening to detect and treat thyroid dysfunction early, especially in young adults transplanted during childhood or adolescence.

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BIOSIMILAR G-CSF IN POST AUTOLOGOUS STEM CELL TRANSPLANTATION SETTING: PRELIMINARY RESULTS

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Background. G-CSF is used in neutropenic phase post-high-dose chemotherapy to reduce infections and support engraftment. Aims. Nowadays biosimilar filgrastim products are available as Tevagraslim resulted safe and effective in autologous transplantation for haematological malignancies are lacking. Methods. In our Institution from November 2010 and February 2011 15 patients underwent autologous peripheral stem cell transplantation for haematological malignancies (8 Non Hodgkin Lymphoma, 1 Hodgkin Lymphoma, 6 Multiple Myeloma). 13 patients received BEAM +/- Rituximab conditioning regimen and 2 pts conditioning regimen as cyclosporine or alentumuzumab. Our patient didn’t received any drug apart from the conditioning regimen itself and the standard prophylaxis. Interestingly the skin rash disappeared, including the skin rash. Conclusions: GVHD is a matter of concern in AloSCT. Few reports in the literature has described a role of certain immunosuppressant drugs given during the period of concern as AloSCT. We discuss an autologous stem cell transplantation with similar efficacy versus other registered regimens treated with originator G-CSF before starting with biosimilar use. The median age was 61 (range 17-76). A median dose of 4.2 (range 2.2-3.85) x 10^6 CD34(+)cells/kg was infused. On the fifth day after progenitor cells infusion, tevagraslim treatment was initiated in all patients by subcutaneous injection. The patients were evaluable as responder or non-responder based on clinical criteria. Results. No adverse events were registered. Administration of tevagraslim was well tolerated in a median of 11 days (range 7-15) while platelets recovery was observed in a median of 12 days (range 7-22). Nine patients (60%) experienced febrile neutropenia resolved with endovenous broad spectrum antibiotics. No differences were reported in terms of white blood cells and platelets recovery among patients receiving biosimilar G-CSF versus originator. Conclusions. We report the preliminary experience Tevagraslim resulted safe and effective in autologous stem cell transplantation with similar efficacy versus other registered growth factors. Considering that every patient received at least 10 days of growth factor support, the use of biosimilar G-CSF may lead to a cost reduction of about 200 € (approximately 50%).

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VIRAL INFECTIONS - STILL A CONSTANT RISK IN PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. Viral infections remain an important cause of morbidity and mortality after hematopoietic stem cell transplantation (HSCT). Aims: to evaluate the frequency and type of viral infections after HSCT.
Methods: We performed a retrospective descriptive unicentric survey in Timisoara BMT Unit during 2001-2010, on 166 consecutive transplants: autologous - 127 and allogeneic - 39 with patients mean age 28,04+/-
16,81 years. Their pathologic background consisted of leukemia-24,09%, malignant lymphoma-42,77%, aplastic anemia-1,8%, solid tumors-13,25% and others-18,07%. All of them had an identical sup-
portive. A pretransplant serological screening for HIV, hepatitis, CMV, VZV, EBV and HHV was undertaken. Complex investigations were per-
formed in febrile neutropenia, graft failure or in symptomatic patients. Results. We registered 49 episodes of documented viral infections, either of exogenous (34,69%) or endogenous (65,3%) origin. Their timing was early-7,5%, intermediate-40% and late phase 52,5%. Seven patients experienced more than one viral infection: one-CMV, poliomyelitis and VZV, VZV, poliomyelitis-2, one-CMV and poliomyelitis-1 and in 6 patients the HBV infection occurred also VZV reactivation. The reactiva-
tion or infection with herpesviruses families happened in 34 cases: CMV-
VZV-28 and HHV6-1. Five patients presented CMV reactivation from which 80% before day+100. CMV appeared more often among the recipients of allogeneic grafts. None of the patients presented clinical signs but all cases were associated with elevated serum levels of hepat-
ic enzymes and with moderate or severe neutropenia. All cases were treated with iv ganciclovir for a median of 3 months and experienced a negativation of CMV-PCR after a median of 2 weeks of therapy. All VZV reactivations/infections appeared after cessation of prophylaxis with acyclovir. 92,85% of patients were VZV-IgG seropositive before HSCT. The cutaneous presentation was: disseminated in 2 patients, extended in two dermatomes in 2 cases and limited to one dermatome in 24 patients. All cases responded to oral acyclovir treatment none of them requiring hospitalization. Post-herpetic neuralgia occurred in 25% of cases. HHV-6 was detected by PCR at day +16 in one patient who pre-
presented a decrease of lymphocytes. The evolution was ful-
minant to hepatic failure despite intensive antiviral treatment. We also identified adenovirus and poliomyelitis infections in 2 and respectively 4 cases. Viruses were detected by PCR in urine samples and the clinical expression was hematuria. A concerning aspect was constituted by hepatic viruses infections found in 9 cases: HBV-5 and HCV-1. In one case the virus was a precore mutant type and in the rest of patients the HBV was wild type. None of the patients developed hepatic failure or died from HBV complications. Conclusions. In the structurally and func-
tionally immunodepressed HSCT patients, viral infections continue to be a life threatening risk. There are eviden limits of proven diagnosis in viral infections and a consequently high proportion of prophylactic and preemptive therapies. Early identification of infections, a specifically tailored therapy and an increased accessibility to diagnostic tools for viral diseases are only some conditions for improvement of life expectancy and increasing of cure rates.

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ANALYSIS OF CD31 AND CD62 DISPARITIES IN HLA-IDENTICAL SIB-
LING CORD BLOOD TRANSPLANTATION FOR HEMOGLOBINOPATHIES. PRELIMINARY DATA
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Nowadays, cord blood transplantation (CBT) represents an effective treatment for several malignant and non malignant haematological dis-
esases. Substantial evidences have been accumulated indicating that HLA donor/recipient compatibility primarily influences the transplantation outcome. However, a little is known about the role of histocompatibility antigens (mHAgs) mismatches in engraftment. Compared to other stem cell sources, a CBT recipient may have a decreased probability of engraftment and a delay in neutrophil and platelet recovery. Platelet endothelial cell adhesion molecule-1 (PECAM-1, or CD31) and leukocyte endothelial adhesion molecule-1 (LECAM-1 or CD62L) are polymorphic proteins that can potentially immunogenic small peptides by intracellular cleavage. These mol-
ecules act as mHAgs and may activate alloreactive T lymphocytes in the transplantation setting. CD31 is expressed on several cell subsets includ-
ing platelets and most leukocytes and is required for the transendothe-


The CBs characteristics are shown in the table. The patients were homogeneous for age, weight, diagnosis (10 thalassemia and one sick-


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EXTENSIVE FASCIITIS AS LATE MANIFESTATION OF GRAFT-VERSUS-
HOST DISEASE, A PURPOSE OF A CASE
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INTRODUCTION: The cGVHD is a multisystem disease that occurs from the day +100 post transplant on and generates inflammatory phe-


PATIENT: Female 18 years old, diagnosed with T Acute Lymphoblas-

In the
days: +43 post-transplant dyschromia skin rash arms (virus PCR negative) appeared; +66: loss of nails; +75: increases in liver enzymes and acne; +103: From day +100 on, the patient received CSA alternating with prednisone 30 mg. On day +240 CSA treatment was deleted and maintained with steroids, starting his reduction in day +380 and its total suspension at day +480 post-transplant. In the month +19: stiffness of small joints of the fingers, elbows and knees and orange peel in the lower extremities, moreover, waxy skin and underlying tissues attached to his forearms. It is suspected as possible scleroderma plates cGVHD compatible. Skin biopsy is performed with a normal and non-inflammatory infiltrate result. Due to the fact that myositis or fasciitis was suspected it was performed: MRI demonstrating an inflammatory involvement of the fascia and septa interfascial, with extension to adjacent muscle fibers, discrete subcutaneous tissue involvement. Elastography: technique based on real time ultrasounds, with colour-coded images, evaluating elasticity of soft tissue stiffness, which discarded muscle involvement (Figure 1).

The results of nerve conduction, electromyography and muscle enzymes were normal. In this way extensive fasciitis as manifestations of cGVHD is diagnosed to the patient and she is on therapy with steroids and mycophenolate mofetil combined with PUVA. Nine months after a new MRI and elastographic exam showed an improvement of lesions mainly in upper arms. Conclusions: The fasciitis is a rare complication in transplanted patients. The diagnosis can be obtained by biopsy, including skin, subcutaneous tissue, fascia and muscle, or alternatively with MRI data supported by other laboratory and / or symptoms. Elastography is a novel technique, useful to assess muscle damage in these cases. It is very important early treatment but we do not know any specific one. Support measures and infectious prophylaxis to prevent deterioration of the quality of life are also needed.

**1491**

**CAN WE USE AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION AS AN INTENSIVE CONSOLIDATION THERAPY FOR ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA PATIENTS IN REMISSION**

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Background: Despite advances in our understanding of its pathogenesis, acute myeloblastic leukemia remains difficult to treat. During the past several decades, improvements in chemotherapeutic regimens and supportive care have resulted in significant but modest progress in treating AML. Conventional chemotherapy is highly effective in the treatment of acute myeloblastic leukemia (AML). About 50-80% of adult patients with de novo acute myeloblastic leukemia achieve complete remission (CR) with currently available chemotherapy regimens consisting of antracyclines and cytarabine. Although initial complete remission can be achieved in a high percentage of patients, relapse occurs in 70-80% of the patients. Two main approaches have been the attempt to eradicate the leukemic clonal cells population via chemotherapy or without autologous stem cell rescue or to pursue a combined approach using an antileukemic therapy combined with an antileukemic immune response via allogeneic bone marrow transplantation. Autologous transplantation compares favorably against allogeneic bone marrow transplant in several ways. Autologous transplantation can be used as a consolidation therapy in the older population, and lack of a matched donor does not preclude the patients from this treatment. Aim: To evaluate autologous hematopoietic stem cell transplantation as an intensive consolidation therapy in adults with acute myeloblastic leukemia in remission. Methods: We report a retrospective analysis on 48 patients diagnosed with de novo AML, who did not have an available histocompatible donor or to pursue a combined approach. Nine months after a new MRI and elastographic exam showed an improvement of lesions mainly in upper arms. Conclusions: The fasciitis is a rare complication in transplanted patients. The diagnosis can be obtained by biopsy, including skin, subcutaneous tissue, fascia and muscle, or alternatively with MRI data supported by other laboratory and / or symptoms. Elastography is a novel technique, useful to assess muscle damage in these cases. It is very important early treatment but we do not know any specific one. Support measures and infectious prophylaxis to prevent deterioration of the quality of life are also needed.

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**OUTCOME OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION FOR ACUTE MYELOGENOUS LEUKEMIA: A SINGLE CENTER RETROSPECTIVE ANALYSIS**


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Background: The selection of patients (pts) upon the best stratification and the timing of transplantation are the key issues of clinical management in AML. Aim: We evaluated the outcome of all pts allografted consecutively for AML in our BMT-unit during 1991-2009. Methods: The data of 149 pts were analyzed. Donors were siblings (107), relatives (11), unrelated (25), double cord blood (CB) (1), haploidentical (5). Thirty-six pts aged 55 (6-80) were transplanted in CR1 after myeloablative (MA) (9), non-myeloablative (NMA) regimen (3) and related-/haplo-CB (5). BEAM (6) and BuCyMel was used in the remaining 2 pts. We used bone marrow as primary source of stem cells in 18 patients, and peripheral blood stem cells in remaining 30 patients. Results: The five years overall survival was 52% and the 5 years disease progression free survival were 42%. We analyzed seven factors that can influence the overall survival and the disease free survival such as: age, disease status, stem cell source, chemotherapies regimens prior to transplantation, conditioning regimens, number of mobilized stem cells. Advanced age and bone marrow stem cell source seems to be more influential factors. We report that the clinical results of autologous hematopoietic stem cell transplantation are sufficiently encouraging to warrant future trials that include autologous transplantation as an option for appropriately selected patients with AML in CR1. We conclude that autologous hematopoietic stem cell transplantation is a reasonable and save intensive consolidation for patients with acute myeloblastic leukemia who do not have a suitable HLA -matched donor.
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IMPROVED OUTCOME IN PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA POST HIGH DOSE CHEMOTHERAPY WITH THE COMBINATION OF BCNU, CYTARABINE, MELPHALAN AND AUTLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION
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Background: Autologous hematopoietic cell transplantation for selected patients (pts) with acute myelogenous leukemia is a viable and reliable option of therapy, even in the era of alternative or reduced intensity transplants. The main advantage of the procedure is very low toxicity and treatment related mortality rate (TRM). Aim: We evaluated the outcome of autologous aML in CR1 in our BMT-un. Methods: In this retrospective study we analyzed the data of 51 pts, aged 37 (12-54) years with de novo AML (49) and secondary AML (2) auto-transplanted in CR1. The conditioning regimens used were: a) BEAM modified (marrow harvest on day-3, infusion of BCNU day-3, etoposide and Cytarabine day-2, Melphalan day-1) and fresh marrow graft (20), b) BUCY-2 (10), c) BUCY-4 (6) and d) BCNU-AraC-Mel (BCNU 300 mg/m² day-3, Cytarabine 3 gr/m²/212 hours day-2, Melphalan 140 mg/m² day-2) (15) with cryopreserved graft either marrow (14) or peripheral blood (PB) (17). The PB graft was mobilized post high dose Etoposide (1,6gr/m²) and has been used in the recent period 2000-2009. Results: For the whole cohort of 51 pts auto-transplanted form 1987-2009 the probability of DFS and OS was 28% and 33% respectively with a Δm follow-up 15 (9-20) years and TRM 6%. In terms of the time of transplant for the early period (1987-1999) the DFS rate was 27%, relapse rate 57% and TRM 10% at 21 years. On the contrary, the OS and DFS of pts who received the BAC regimen and mobilized PB as graft during the late period 2000-2009 was 53% and 53% respectively, while the TRM was 0% at 8 years. Interestingly, pts with intermediate risk group cyto genetic (17), mostly normal karyotype, succeeded rates of OS 82% and DFS 57% at 8 years. Summary/conclusions: The challenge of refinements of clinical management of patients with aML is to identify individual patients likely to benefit from specific therapeutic modalities, such as autologous hematopoietic cell transplantation, as an intensified consolidation treatment with zero mortality rate. It can be employed to maintain the remission and cure the disease. Better supportive care has enhanced the ability of nearly each aML patient to deliver high dose chemotherapy plus autologous rescue with mobilized PB graft. Moreover, comparison of various used conditioning regimens demonstrated a DFS survival advantage of BEAM (28%) over the others, i.e modified BEAM (32%) or BUCY-2, BUCY-4 (31%) at 7, 21 and 18 years respectively.

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SUCCESSFUL TRANSFUSION-FREE ALLOGENEIC STEM CELL TRANSPLANTATION IN A JEHOVA’S WITNESS PATIENT: A CASE REPORT
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Background: Allogeneic stem cell transplantation (AloSCT) is a curative approach for patients with relapsed acute lymphoblastic leukemia (ALL). Less intensive modalities of the conditioning regimens have arisen to decrease the risk of toxicity. We describe a case of a patient with a relapsed ALL who proceeded to a nonmyeloablative AloSCT from her HLA matched sibling without any transfusion support as a consequence of her denomination as Jehovah’s Witnesses. Case report: A 35 years old lady was diagnosed with pre-B ALL in July 2007 without adverse cytogenetics. She refused any transfusion support during the chemotherapy treatment, which led to a lesser intensive induction chemotherapy mainly to minimize the risk of bleeding by thrombocytopenia albeit a higher risk of relapse. After induction chemotherapy with vincristine, daunorubicine and predinose she achieved a complete remission (CR) and was started in maintenance therapy (MTT) for two years. On march 2010 she relapsed and was retreated with the same induction chemotherapy with a minimal residual disease (MRD) of 2% by flow cytometry in the bone marrow samples. Thereafter she received new MTT until august 2010 when she overt relapsed again. Rescue chemotherapy with vinblastine, cytarabine and VP-16 was administered achieving CR again with negative MRD. On October 2010 she underwent a nonmyeloablative AloSCT from a matched sibling. We adopted three strategies to prevent severe cytopenias during the AloSCT: firstly we administered a reduced intensity conditioning with total doses of 6.4 mg/kg iv busulfan, 1000 mg/m² iv Cyclofosfamide and 90 mg/m² iv Fludarabine. Secondly, we performed a larger than usual graft infusion of mobilized peripheral blood stem cell CD34+ cell from her donor (total dose of 7.75x10⁶ /kgr) by three consecutive collection dates. The collected cells were infused “fresh” at the evening of the same collection day. Total CD3 infused cells were 5x10⁶/kgr. Finally we used three different human recombinants growth factors to improve the engraftment: erythropoietin 10.000 UI three times a week from one month before the AloSCT, G-CSF 300 µgr daily from d+6 to d+13 and romiplostim 5 µgr/kgr 2 doses on d+5 and d+10. Neutrophil engraftment occurred on d+13. Platelet engraftment occurred on d+14. No significant bleeding occurred (platelet nadir of 31x10³/ul on d+12). She was discharged on d+14 and has remained as an outpatient up to date. No one single blood product has been infused to the patient since her diagnosis of ALL. Currently she’s alive in CR on d+125, with mixed chimerism and on treatment for grade II graft versus host disease. Conclusion; Transfusion-free stem cell transplantation has been described in isolated cases in the literature. New available second generations thrombopoietic growth factors like romiplostim, allows a safer approach to this procedure. We conclude that transfusion-free AloSCT is feasible.

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LEVOFLOXACIN FOR BK VIRUS-ASSOCIATED HEMORRHAGIC CYSTITIS IN THE POST-HEMATOPOIETIC STEM CELL TRANSPLANTATION PERIOD
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Background: BK virus (BKV) is one of the most common causative agents of hemorrhagic cystitis (HC) in the early postengraftment period of hematopoietic stem cell transplantation (HSCT). Ciclosporin may be a potentially effective treatment option with a 67% complete response rate. Unfortunately, it is not available in most European countries. High cost rate and possible renal side effects are the other important disadvantages of Ciclosporin. In vitro activity of Fluoroquinolones has long been known. But their in vivo effects are limited. There are no data about the efficacy of respiratory fluoroquinolones such as levofloxacin. Aim: We present here the clinical and molecular activity of levofloxacin in three patients with BKV-related HC experienced during postengraftment period following HSCT. Methods: Patients’ chart records were reviewed retrospectively.

Results: Data about the three patients who experienced severe HC within the first 100 days of HSCT were reviewed (Table). All patients needed continuous intravesical irrigation because of gross hematuria and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Analyses for adenovirus, cytomegalovirus, mycoplasma, and ureaplasma and urinary obstruction caused by blood clots. Routine microscopic analyses and bacterial cultures revealed no specific pathogenic organism. Ana...
ma were all negative. Patients were receiving immunosuppressive agents in appropriate doses. None of the patients had any clinical sign of graft versus host disease. But BKV was detected in the urine of patients in varying degrees. All patients received ciprofloxacin at least two weeks and intravesical risperidone. Additionally, hyperbaric oxygen and subsequently external iliac artery embolization were performed in patient 3. But no improvement was observed. Patient 1 received levofloxacin, 500 mg qd, his HC resolved completely in one week. The other two patients also received the same treatment and complete clinical response was achieved in both. Levofloxacin treatment was given for 8 weeks and urine BKV copies were monitored. BKV copies in urine decreased more than 90% in all (Figure). These patients are still disease- and GVHD-free and alive.

Leukapheresis was given to the other patient who had HC not related to BK within the 100 days of post-HSCT period. But no response was observed. Conclusions: Leukapheresis may be an effective treatment option in patients with refractory BKV-related HC experienced during post-HSCT period. Efficacy of levofloxacin may be associated with the inhibition of DNA topoisomerase IV and DNA gyrase. Some other factors specific to levofloxacin may have an effect on BKV.

1496

LARGE VOLUME LEUKapheresis (LVl) FOR PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) COLLECTION IN HEALTHY DONORS FOR ALLOGENEIC transplantation: EXPERIENCE OF A SINGLE CENTER

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Introduction: There are no defined guidelines to improve the efficiency of collections of peripheral blood cells in allogeneic transplant programmes. We present our experience using Large Volume leukapheresis (defined as more than 5 times the total blood volume) for collection of PBPC in healthy donors, applied in our centre with the aim of simplification of procedure. Methods and donors: 124 healthy donors (66 males and 58 females; median age 48, 19-73) were included in this study since November 2001 to December 2010. All donors received 12-14 g/kg/day s.c of rhG-CSF (filgrastim®, Amgen, Thousand Oaks, CA, USA) in two doses during 4 days for mobilization. Our main objective was to yield at least 3x10^5/kg receptor weigh of CD 34+ cells. Leukapheresis was started on the fifth day after the administration of rhG-CSF. Large Volume Leukapheresis (LVl) was programmed and cells were collected through peripheral vein access in most cases, using a Cobe Spectra separator (COBE SPECTRA, Gambro BCT, Lakewood, CO, USA). Intra-process controls were performed to finish the procedure according the objectives and results. Results: The median number of patient’s blood volumes processed was 3 L (1-4). 113 donors (92%) required only one session to achieve the CD34+ cells objective. PBPC collection yield a median of 6.02x10^6/kg CD 34+ cells (2.85-13.57). No mobilization failure was observed. All products were transplanted with rapid and sustained engraftment in all cases. No serious adverse effects were observed and no infections related to the PBPC collection were observed. Efficacy and reversibility. Conclusions: In our experience Large Volume Leukapheresis (LVl) allows an adequate PBPC collection for transplantation with the reversibility and a fast engraftment.

1497

METHYLATION STATUS OF RUNX2, OSX, DLX5 AND BSP GENE PROMOTERS IN OSTEOSTOMATIC DIFFERENTIATION OF MESENCHYMAL STEM CELLS (MSCs)

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Background. Epigenetic mechanisms, including DNA methylation, histone modifications, and non-coding RNA-mediated regulatory events, are essential to controlling the heritable cellular memory of gene expression during differentiation. DNA methylation is modulating gene expression by addition of a methyl moiety to the cytosine-5 position in CpG islands. Two specific osteoblast transcription factors and one non-specific transcription factor i.e. RUNX2, OSX and DLX5 are important in regulating osteoblast related genes as well as osteocalcin, BSP, OPN and collagen type Iα1. Aims: In this research we speculated whether epigenetic regulation of these genes plays any role in osteoblastic differentiation of mesenchymal stem cells. Therefore, we evaluated methylation status of RUNX2, OSX, DLX5 and BSP promoters in osteoblastic differentiation of MSCs. Materials and Methods: MSCs were isolated from human bone marrow (a written informed consent was obtained from all participants). Osteogenic differentiation was done under the influence of osteogenic medium containing 100 nM of dexamethasone, 50 μM of ascorbate, and in the presence of 10% FCS. DNA and RNA extraction were carried out after the first, second and third weeks of culture and also from undifferentiated MSCs. After DNA extraction and bisulfate treatment, gene specific methylation analysis for RUNX2, OSX, DLX5 and BSP was carried out using methylation specific PCR (MSP). Moreover, quantitative RT-PCR was used to measure the gene expression. Results: MSP analysis revealed that promoter methylation status didn’t change in RUNX2, DLX5 and BSP promoters during osteoblastic differentiation of MSCs. In contrast, OSX promoter showed a dynamic change of methylation pattern while MSCs were gradually differentiated to osteoblasts. Conclusions: RUNX2, OSX, DLX5 and BSP promoter regions showed 3 different methylation patterns during MSCs differentiation. This study shed light on the osteoblastic differentiation of MSCs by showing dynamism in methylation change during this process.

1498

HEMATOLOGIC ABNORMALITIES AND ETIOLOGICAL FACTORS OF OCCUPATIONAL TOXIC CYTOPENIAS, ABOUT 138 CASES

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Background: innovations in the fields of industry and research and the proliferation of applications of chemistry explain the growth of the use of chemicals to which humans are exposed both in the workplace than in domestic. Exposure to these xenobiotics may be responsible for a blood-toxicity. Aims: We are interested in studying abnormal peripheral blood findings of toxic origin in the work environment and factors in the workplace. Methods: This is a retrospective study over a period of 5 years (January 2004-December 2008) on cases of cytopenia collected from results with professional activity, followed by the hematology department of university hospital Hedi Chaker, Sfax, Tunisia and professional investigation which was conducted by the department of occupational medicine at the same hospital. Results: A total of 5611 patient records identified the number of patients with suspected toxic cytopenia is 138. The annual recruitment of hematology department of Sfax is 77 cases of presumed toxic origin cytopenia year. The prevalence is estimated at 7%. A male predominance is noted with a sex ratio of 1.76. The average age is 40 years. Nearly a third of the population belongs to the age of 25 to 34 years. A monocytopenia is found in 70% of cases with leukopenia 40% of cases, thrombocytopenia 27% and 3% of anemia cases. The bone marrow examination was performed in only 34 patients. Ten patients marrow in 33 cases, poor in 6 cases and dysplastic in 4 cases Our patients perform in diverse industries, manufacturing is the leader with a rate of 49% followed by the services sector with a rate of 32% then 10% agriculture, building industry and public works 5%, 4% heavy industry. Three quarters of patients are workers. Professionals in agriculture are the most represented. Organic solvents, mainly benzene are the class of nuisance most reported in our series. Conclusions: In our series and in literature, the main industries using benzene are the chemical industries of plastic, mechanical Petrochemicals. In the literature, very few data are available about the incidence of cytopenias toxic. Male predominance and young age of the patients are found both in our work in literature.

1499

SELF-RENEWAL ABILITY OF MARKED BY LENTIVIRAL VECTOR MESENCHYMAL STEM CELLS

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Background. Stem cells have to be able to differentiate and self-renew. Mesenchymal stem cells (MSCs) form hematopoietic microenvironment in vitro in long-term bone marrow culture (LTBMC), while in vivo this ability could be analyzed by implantation of femur bone marrow plug or adherent cell layer (ACL) from LTBMC under renal capsule of synchronous recipients. Stromal cells in such foci are derived from donor MSCs while hematopoietic cells have recipient's origin. The size of the foci formed is proportional to femur equivalent transplanted and can be used for semi-quantitative determination of MSC number. The golden stan-
dard for stem cells is their ability to self-renew which could be proved by retransplantation. After the retransplantation all differentiated stromal cells in the hematopoietic foci are lost and the microenvironment in newly formed foci develops from transplanted MSCs completely de novo. Aims. The aim of the study was to investigate the self-renewal ability of marked murine MSCs able to transfer hematopoietic microenvironment. Methods. ACLs of 2-week-old LTBMSCs were infected with 100 ml of concentrated (108 viral particles/ml) self-inactivating HIV LeGö vector encoding EGFP (all plasmids and Phoenix cells were provided by Prof. Boris Fehe) in 3 ml of aMEM with 10% FCS and 8 mg/ml polybrene for 6 hours. In 2 weeks after the infection scraped by rubber policemen ACLs were implanted under the renal capsule of syngeneic mice. Colony-forming units fibroblast (CFU-Fs) were analyzed by plating 20000 nucleated cells from the focus per well of 96-well plate in aMEM supplemented with 20% FCS and 5 ng/ml FGF2. In 6 weeks the number of marked CFU-Fs was measured in 4 foci formed and other foci were retransplanted under the renal capsule of secondary recipients. The procedures were repeated 4 times and the numbers of marked CFU-Fs were measured in 4 foci after each retransplantation.

Results. The proportion of marked cells in ACLs before transplantation under the renal capsule was 5.3 ± 0.6 %. The size of the foci formed was stable throughout all retransplantations, the marked CFU-Fs were revealed in all foci (table). All ossicles in the foci formed were EGFP positive.

Conclusions. As CFU-Fs are the progeny of MSCs, the availability of genetically marked CFU-Fs in the foci points to the presence of marked MSCs. Obviously marked MSCs are able to transfer hematopoietic microenvironment at least 5 times and keep the ability to produce the considerable number of marked progeny, their ability to self-renew is proved. Thus, all crucial MSCs features (prolonged lifespan, ability to self-renew and differentiate) are not affected by stable integration of the vector containing gene of interest meaning that these cells are preferable targets for gene therapy.

1500 INCREASED LEVELS OF BONE MARROW ENDOTHELIAL CELLS, PROGENITOR AND MATURE, IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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The role of endothelial progenitors (EPCs) and mature cells (ECs) in angiogenesis has gained the scientific interest of many recent studies. Aim: The immunophenotypic analysis of EC subpopulations in the bone marrow (BM) and the study of their potential role in the pathogenesis of various malignant and autoimmune diseases. Methods: Bone marrow cells from children with acute lymphoblastic leukemia at diagnosis (ALLd, n=9), at day 15 (ALL15d, n=5), and day 33 of treatment remission is achieved (ALL33d, n=7), ALL under consolidation therapy (ALLct, n=10), ALL following the end of treatment (ALLet, n=10), solid tumors without BM involvement at diagnosis (ST, n=9), idiopathic thrombocytopenic purpura (ITP, n=4) and autoimmune diseases (AI, n=5) were studied. The putative antigenic phenotypes of EPCs and ECs were tested for the expression of specific surface markers, their ability to differentiate into adipocytes and osteoblasts. In addition, SDS-MSCs drastically decreased the mitogen-induced lymphocyte proliferation, in a dose dependent manner. We also cultured neutrophils obtained from HD in presence or absence of MSCs at different time points. We demonstrated that SDS-MSCs were comparable to HD-MSCs in supporting the viability of neutrophils. Importantly, SDS-MSCs were able to produce high amount of IL-6 (mean= 2658 pg/ml, range= 2086-3229 pg/ml), a crucial cytokine involved in the protection of neutrophils from apoptosis. Results: SDS-MSCs derived from SDS patients (SDS-MSCs) displayed typical fibroblastoid morphology; they were consistently devoid of contaminating hematopoietic cells, being negative for CD34, CD45, HLA-DR, CD11b, CD19, and CD14, but expressed common MSC markers including CD90, CD73, CD105 and HLA-ABC. Similarly to MSCs obtained from healthy donors (HD-MSCs), these cells were able to differentiate into adipocytes and osteoblasts. In addition, SDS-MSCs drastically decreased the mitogen-induced lymphocyte proliferation, in a dose dependent manner. We also cultured neutrophils obtained from HD in presence or absence of MSCs at different time points. We demonstrated that SDS-MSCs were comparable to HD-MSCs in supporting the viability of neutrophils. Importantly, SDS-MSCs were able to produce high amount of IL-6 (mean= 2658 pg/ml, range= 2086-3229 pg/ml), a crucial cytokine involved in the protection of neutrophils from apoptosis.

Conclusions: As CFU-Fs are the progeny of MSCs, the availability of genetically marked CFU-Fs in the foci points to the presence of marked MSCs. Obviously marked MSCs are able to transfer hematopoietic microenvironment at least 5 times and keep the ability to produce the considerable number of marked progeny, their ability to self-renew is proved. Thus, all crucial MSCs features (prolonged lifespan, ability to self-renew and differentiate) are not affected by stable integration of the vector containing gene of interest meaning that these cells are preferable targets for gene therapy.
**Background and Aims:** We recently reported that acute myelogenous leukemia blasts and chronic myelogenous leukemia cells can convert to stromal myofibroblasts to create an environment for the proliferation of leukemic cells in vitro and also in vivo in the immunodeficiency murine bone marrow. In normal hematopoiesis, hematopoietic cells are also speculated to contribute to the formation of the hematopoietic stromal tissue. And we also hypothesized that bone marrow stromal myofibroblasts may convert to hematopoietic cells. To make clear this issue, bone marrow stromal myofibroblasts from informed healthy volunteers were cultured with phytohemagglutinin (PHA-P)-stimulated lymphocyte conditioned medium in vitro. After one week morphological changes were observed microscopically, in which budding from the spindle-shaped fibroblast was demonstrated, and a few cells were detached from the dishes and were floating. To determine the mechanism of this biological finding, expression cloning was performed. Materials and methods. Blood was collected from the informed healthy volunteers, and lymphocyte-rich fraction was separated with gravity sedimentation method. PHA-P was added in the cultures, and after 48 hours lymphocytes were collected, with which cdNA library was constructed after ligation to the mammalian expression vector. Electroporation to Ecoli DH10β cells made 5x105 independent colonies, which were divided into sub-pools (1000 independent colonies/pool). Plasmids were prepared from each pool and transfected to COS7 cells with DEAE-Dextran method. After 5 days of culture supernatants were collected, which were used until use. Bone marrow cells were aspirated from informed healthy individuals, and were separated to mononuclear cells. Adherent cells were cultured after 2-hour cultures in the coated dishes. Cells were further cultured long term with splitting using trypsin/EDTA once a week. After one month, bone marrow-derived STRO-1(+) and smooth muscle actin (+) myofibroblasts were prepared as a target for the expression cloning. The transfected COS7 supernatants were added in the myofibroblast-cultures (final 10%), and cells were further cultured for one week. RNA was extracted from the cultured cells, and cdNA was synthesized. Positive clones were selected with reverse transcription-polymerase chain reaction using human CD34 primers, and further selected to be a single clone. Results and discussion: Isolated single clone was revealed to be human interleukin 1β. When the purified interleukin 1β protein was added in the myofibroblast cultures, cell growth was increased, and up-regulation of the expression of several hematopoietic cytokine receptors including c-kit was observed. Now we determine the precise actions of human interleukin 1β on the reprogramming of bone marrow stroma-derived myofibroblasts toward hematopoietic stem cells.

1503

**BONE MARROW MESENCHYMAL STROMAL CELLS PROMOTE PROLIFERATION OF LYMPHOMA CELLS AND GASTRIC CANCER CELLS VIA CELL-TYPE SPECIFIC MANNER**

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Background: Bone-marrow derived mesenchymal stromal cell (bM-SC), one of major sources of MSC, has been known to participate for reconstituting microenvironment. Cancer cells, as like normal cells, have been known to need microenvironmental support for their survival and growth. Studies show that MSCs were recruited and integrated into tumors, however, what is the precise effect of MSC on tumor cells still remains unclear. Aims: To address this, investigators employed coculture and mice co-transplantation assay with hematopoietic tissue-derived bM-SC and adipose tissue-derived MSC (ADSC), and hematologic and malignancies (lymphoma cells and gastric cancer cells). Methods: Lymphoma cells (Pfeiffer and Raji) and gastric cancer cells (SNU5FU and SNUADR) were cultured in RPMI 1640 with basic supplements. Mice were xenotransplanted with the cell lines. Evans blue method or flowcytometry with propidium iodide and Annexin V. Cancer cells (2x105) only or mixed with MSC (5x104) were transplanted into NOG/SCID mice (13-15 week-old) subcutaneously. Results: BmMSC increased the proliferation of Pfeiffer cells by direct contact (x3.5), but not by indirect contact compared with Pfeiffer alone at day 4. BmMSC also promoted the proliferation of SNU5FU cells by both direct (x1.9) and indirect (x1.6) contact at day 6. Whereas, Raji cells and SNUADR cells were minimally influence. BmMSC-cocultured Pfeiffer showed increased cell viability (16.7%) compared with Pfeiffer alone (9.8%) under the doxorubicin treatment. For the SNU5FU cell under 5-Fluorouracil treatment, two different bmMSC didn’t show chemoprotective effect, but ADSC-cocultured cells showed increased chemoresistance (84.1%) compared with SNU5FU alone (46.7%). In NOG/SCID mice cotransplantation assay, SNU5FU alone, SNU5FU-bmMSC and SNU5FU-ADSC, cotransplanted mice showed 78% (3/4) tumor formation, respectively. SNU5FU-alone induced tumor and SNU5FU-bmMSC induced tumor showed compact tumor mass without desmoplasia, whereas SNU5FU-ADSC tumor showed marked stromal reaction, infiltrative growth and lung metastasis. Summary/Conclusions: BmMSC showed growth promoting effect on Pfeiffer and SNU5FU, but not on Raji. BmMSC showed chemoprotective effect on Pfeiffer cells, but not on SNU5FU cells. ADSC, but not bmMSC, showed in-vitro chemoprotection and in-vivo tumor promoting effect on SNU5FU. These findings suggest that the MSC type, the targeted tumor cell type and environmental condition should be considered for understanding the effect of MSC on tumor cells.

1504

**POOR CLINICAL OUTCOMES IN NON TRANSFUSED PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH)**

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Introduction. PNH is a debilitating and life-threatening hematopoietic stem cell disorder characterized by chronic hemolysis leading to significant morbidity, end organ damage and mortality in PNH patients. Historically, PNH was considered an anemic disease with management focused on improving hemoglobin (Hb) levels through transfusions. This has led to the perception by some that absence of transfusion requirements or mild anemia as a measure of mild or stable disease. Aims. To understand the impact of transfusions on the risk factors associated with poor patient outcome, we retrospectively analyzed medical charts of 123 PNH patients with no documentation of transfusions from national data registry in South Korea over the last 41 years. Results Patient ages ranged from 17 to 88 years (median 37 years), median PNH duration was 6.8 years (14 days to 27 years), and median PNH granulocyte count was 40% and median LDH at diagnosis was 1.3 above normal. Evaluating hemolytic symptoms associated with end organ damage, we found that approximately 12% of patients with no documentation of transfusions had late stage renal dysfunction (defined as history of renal failure or GFR <60 ml/min/1.73 m2), a predictor of early mortality. Gastrointestinal pain, a symptom predictive of thrombosis and poor quality of life, was reported in 38% of non-transfused patients. Dyspnea, a symptom of pulmonary hypertension and predictive of thrombosis was reported in 28% of patients. Elevated hemolysis at diagnosis (as measured by LDH ≥1.5 above normal) is predictive of early mortality. We evaluated non-transfused patients with LDH ≥1.5 above normal at diagnosis (n=70) or LDH < 1.5 of normal (n=20) for patient death. There were 6 patient deaths reported (median 7.3 yrs disease duration) in the hemolytic non-transfused group compared to no reported deaths (median 4.6 yrs disease duration) in non-transfused patients with no elevated hemolysis at diagnosis. Conclusion: Our data demonstrates that patients who are not transfused suffer from significant disease burden. Elevated hemolysis leads to poor outcomes in PNH patients and should be the target for PNH management.

1505

**TWO CASES OF LANGERHANS HISTOCYTOSIS X AND THEIR THERAPEUTIC APPROACH**

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Background. Langerhans cell histiocytosis results from the abnormal accumulation of dendritic cells of the skin, which proliferate in many organ systems along with lymphocytes, macrophages and eosinophils. There is multisystem disease involvement described with lytic skull lesions, involvement of the skin and lymph nodes. Aims: To present the rare disease of Langerhans histiocytosis.

Methods: Two clinical cases of the rare histiocytosis X cases are reported along with the therapeutic aspects. A 22-year-old woman presented with a 3-month history of headache localised to the right posterior parietal area. The patient reported concurrent migraines and vomiting. A brain MRI revealed a non-enhancing semicircular mass 1,3x0,9 cm of parietal bone with no pressure effects on the nearby structures, meninges and soft tissues. A whole body bone scan was free of disease. Biopsy of the osteolytic lesion revealed lesion similar to Langerhans histiocytosis with eosiophilic granulomatous origin based on positive immunohistochemistry for S-100/Langerin and CD1a (+/-). The histiocytes had medium size with nuclear grooves. The patient had normal lung function, and a negative for disease thorax CT. The abdomen CT was normal with no lymph nodes enlarged. Therapy included complete surgical excision and replacement of the area with bone transplant. She received prednisone 1mg/kg/day for 4 weeks and tapering over a 2-week period followed with a weekly infusion of vinblastine 6mg/m2 for 5 weeks. Then, the same dose of prednisone was administered (5 days/week) followed by vinblastine once every three weeks to complete a 6-month period.

Results: 6-mercaptopurine 30mg/m2 was administered due to desire of future pregnancy. She was previously diagnosed with systemic lupus erythematosus under medication with hydroxychloroquine 200 mg/day from June to September every year. The second patient a 45-year-old female reported to our clinic after a painless palpable right submandibular area. An ultrasonad at this area revealed a multilobular, compact mass 1,9X1,5 cm. The neck CT confirmed the mass as bilobular with central erosion. Later, it was totally excised and sent for biopsy. The biopsy was positive Langerhans cells (S-100+, Langerin+, KP1+, CD30+, CD20+, CD5, S53). Nearby the atypic cells were expressed in lowest levels that were found to be gradually increasing.

Conclusions: Human CML hematopoietic stem cells are not able to survive in the CNS of either normal mice or mice with severe combined immunodeficiency (SCID) background. Rare bcr-abl positive cells could be observed at day seven, but not thereafter. We did not observe colocalization of the bcr-abl signal with betaIII-tubulin or antiGFAP by immunofluorescence. FISH. Neural differentiation was checked by colocalization of the FISH signal with antitbetall-tubulin and antiGFAP by immunofluorescence. Results: The mice received 1 to 2x105 CD34 selected cells. No cells with the bcr-abl translocation could be observed at 7 day post-transplant in either the Swiss or the NOD.Cg-Pkdcsk I2g2tm1Wjl/SzJ mice. In the 6 neonatal transplanted mice some signal (1.3x0.38), all of them were negative(0.26±0.08, p=0.031). At consolidation Fas levels were found to be decreased compared to day33 (16.1±4.18) and were again increased at the end of therapy (ALLd vs ALLet: 8.02±1.94 vs 24.96±7.95, p=0.024).

This abstract has been withdrawn.

FAS AND FAS LIGAND EXPRESSION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA, BENIGN HEMATOLOGICAL DISEASES AND SOLID TUMORS

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Fas/Fasl is a key pathway of cellular apoptosis. Fas receptor is expressed on membranes of both normal and neoplastic cells while, Fas ligand (FasL) is mainly on activated T-lymphocytes. Fas-FasL abnormalities have been detected in malignancies and autoimmune diseases and implicated in resistance to treatment. The aim of the study was the determination of the levels of Fas, Fasl and their coexpression in bone marrow cells of children with acute lymphoblastic leukaemia (ALL) at diagnosis, during the course of treatment and the comparison with relevant levels in other hematological and neoplastic diseases. Methods: Expression levels of Fas and Fasl were determined with flow cytometry in children with ALL at diagnosis (ALLd, n=13), on day 15 of treatment (d15, n=6), on day33 (ALLdSSS3, n=6), during consolidation (ALLhr, n=12) and at the end of therapy (ALLhT, n=7) as well as in children with Langerhans Histiocytosis (LCH, n=4), cytopenias (Cytp, n=8) and solid tumors without bone marrow involvement at diagnosis (STd, n=5) and on therapy (SThT, n=6). Results: The lowest levels of Fas expression were detected at diagnosis of ALL and were gradually statistically significantly increased until remission of day 33 (ALLdSSS3: 8.02±1.94 vs 24.04±6.11, p=0.05). At consolidation Fas levels were found to be decreased compared to day33 (16.1±4.18) and were again increased at the end of therapy (ALLd vs ALLet: 8.02±1.94 vs 24.96±7.95, p=0.024). On the contrary, Fasl levels were gradually decreased and finally increased to similar to diagnosis levels at the end of treatment (ALLd: 4.59±1.41, ALLet: 5.89±1.99). In solid tumors at diagnosis Fas levels were similar to the ones while on chemotherapy (STd vs SThT:16.04±2.2 vs 15.3±6.4). The highest Fas levels were detected in the group of STd with the relevant levels on treatment being lower in comparison (STd vs SThT: 10.91±3.32 vs 29.2±2.07, p=0.052). In LCH both Fas and Fasl levels were found to be as low as at ALL diagnosis. In cytopenias no significant difference was observed between groups for either Fas (11.05±4.49 or Fasl (3.01±0.62). As for Fas+Fasl coexpression no difference was evident between ALLd, ALLet and STd or SThT (ALLd: 0.73±0.38, ALLet: 0.64±0.17, STd: 0.62±0.95, SThT:0.66±0.38) The lowest coexpression levels were observed in the group of cytopenias with statistical significant difference compared to STd (0.68±0.095 vs 0.26±0.08, p=0.051). In conclusion, at diagnosis of ALL Fas levels were expressed in lowest levels that were found to be gradually increased at remission and at the end of treatment. This finding probably correlates with the apoptotic process of the leukemia clone and possibly with response to treatment.

THE ONCOGENE EVI1 ENHANCES TRANSCRIPTIONAL AND PROLIFERATIVE RESPONSES TO ATRA

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Human chronic myeloid leukemia hematopoietic cells do not transdifferentiate into neural lineages

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Introduction. Adult hematopoietic stem cells (HSC) are able to survive in the CNS of adult animals and to transdifferentiate into neural cells upon exposure to signals generated by CNS tissue injury (Bonnilla et al, 2002). This fact has been difficult to prove when studying human HSC in animal models due to the lack of a good marker to follow the fate of the transplanted cells. Chronic myeloid leukemia (CML) is a stem cell disorder characterized by immune-scape and resistance to apoptosis due to an acquired chromosomal translocation, the t(9;22). This translocation generate a specific BCR-ABL chimeric gene that may be identified by Fluorescence in situ hybridization (FISH), and could facilitate tracking the fate of CML human cells in xenogeneic transplant experiments. Methods: We isolated CD34+ positive cells from two untreated chronic phase CML patients and transplanted 105 cells per animal. The CML stem cells were infused in the white matter of the parietal hemisphere of six adult Swiss mice, six neonates and six NOD.Cg-Pkdcsk I2g2tm1Wjl/SzJ immunsuppressed mice. Mice were sacrificed at 7, 15 and 30 days. LS1(9;22) BCR-ABL Dual Fusion Dual Color Translocation Probe (Vysis) was used to confirm the FISH. Neural differentiation was checked by colocalization of the FISH signal with antitbetall-tubulin and antiGFAP by immunofluorescence. Results: The mice received 1 to 2x105 CD34 selected cells. No cells with the bcr-abl translocation could be observed at 7 day post-transplant in either the Swiss or the NOD.Cg-Pkdcsk I2g2tm1Wjl/SzJ mice. In the 6 neonatal transplanted mice some signal (1.3x0.38), all of them were negative(0.26±0.08, p=0.031). At conclusion, at diagnosis of ALL Fas levels were expressed in lowest levels that were found to be gradually increased at remission and at the end of treatment. This finding probably correlates with the apoptotic process of the leukemia clone and possibly with response to treatment.

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Background. EVI1 is an oncogene whose overexpression is associated with a poor prognosis in myeloid leukemias and certain solid tumors. Even though its protein product exhibits properties of a transcription factor, only few direct target genes have been identified. Several reports suggest that EVI1 may elicit its biological effects through modulation of the activity of other transcription factors. In this context we have recently shown that EVI1 affected all-trans retinoic acid (ATRA) dependent gene expression in HL-60 cells. The ATRA induced, a v-erbB, a myc and a erbA gene and counteracted its own upregulation by ATRA. Aims: In the present work we asked whether EVI1 would modulate the ATRA regulation of additional genes and whether it would also affect biological responses elicited by ATRA. Methods. Triplicate cultures of U937 cells with or without ectopic expression of EVI1 were incubated with ATRA or solvent for 24 h. RNA was extracted and subjected to microarray analyses (Affymetrix HG-U133 Plus 2.0). Candidate genes were confirmed by qRT-PCR. Cell cycle analysis was performed by FACS after propidium iodide staining. Results: Array analyses revealed that the transcriptional response to ATRA of 44 unique genes was modulated by EVI1 at least twofold and in a statistically significant manner. The ATRA induction of 34 and the ATRA repression of seven genes was enhanced by EVI1, while ATRA repression of three genes was counteracted by EVI1. Eight genes were selected for confirmation by qRT-PCR. The regulatory pattern observed in the array experiments was confirmed in all cases. To determine whether EVI1 would also enhance biological responses to ATRA, cell cycle analyses were performed. Upon treatment with ATRA a higher percentage of EVI1 overexpressing cells than of empty vector containing cells accumulated in the G0/G1 phase of the cell cycle, demonstrating that indeed EVI1 enhances biological responses elicited by ATRA. Summary/Conclusions. EVI1 enhances the transcriptional and growth inhibitory responses of ATRA in human myeloid cells. It will be interesting to investigate whether AML patients overexpressing EVI1 are responsive to differentiation therapy.

1510
PIPKII-ALFA REGULATES THE ALFA AND GAMMA GLOBINS EXPRESSION IN HEMATOPOETIC-DERIVED CELLS
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Background. Phosphatidylinositol phosphate kinases (PIPK) belong to a family of enzymes that generate various lipid messengers. The PIK family belongs to two major subfamilies, type I (α and β) and type II (α, β and γ). In a recent study in our laboratory, the PIPKII α gene was differentially expressed in reticulocytes from 2 siblings with hemoglobin (Hb) disease. Expressions of both the PIPKII α and β-globin genes were higher in the patient with the higher Hb level, suggesting a possible relationship between PIPKII α and the productions of globins. However, the role of PIPKII α gene in hematopoietic process has been less directly addressed. Aims: To evaluate the PIPKII α expression in hematopoietic-derived cells line and to investigate the effects of PIPKII α silencing in K562 cells on alfa and gama globin expression and on proliferation, cell cycle, differentiation and apoptosis. Methods: K562, KG1, NB4, HL60 and P39 cell lines were used. Specific siRNA-expressing vector targeting the PIPKII α gene or no specific sequence were electroporated in the K562 cell line. Cells expressing no specific sequence or parental cells were used such as control. Quantitative PCR (qPCR) and Western blot analysis were performed to determine the expression of PIPKII α, alfa and gama globin, β actin and GAPDH were used as control in qPCR and actin in Western blot. After 48 hours of culture, proliferation was analyzed by MTT assays, apoptosis by Annexin-V and propidium iodide (PI), cell cycle by incubation with PI and RNase A buffer and flow cytometry. After 15 days of silencing the differentiation was evaluated by glycoporphin A and transferrin fluorescence intensity and percentage of double positive cells. Imatinib was used as control in the arrest of proliferation and induction of apoptosis in K562 cells. Results: qPCR and Western blot showed that PIPKII α was expressed in all the cell lines tested and was observed a slight increase of PIPKII α expression in K562 cells when compared with other cells included in this study. The levels of PIPKII α mRNA and PIPKII γ protein in knockdown cells were significantly reduced by 80% and 75%, respectively (P<0.05). MTT assays showed that the proliferation was slightly reduced by 10% in PIPKII α knockdown cells when compared with control cells (P<0.05). Cell cycle, apoptosis and differentiation analysis showed no difference in PIPKII α knockdown compared when control cells. Interestingly, PIPKII α silencing resulted in a significant increase in alfa and gamma globin expression compared to control cells, as observed by qPCR and Western blot (P<0.05). Conclusions: PIPKII α is express in hematopoietic-derived cells and PIPKII α silencing results in slight decrease in proliferation and an increase of alfa and gama expression. Our findings show that in K562 cells there may be a complex regulatory mechanism that acts on α and γ genes in response to the reduction in PIPKII α gene expression.

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cells were cultured in complete RPMI medium with 10 mili molar of TKIs during four or eight hours. After the experiment, RNA from cell lines were extracted, RNA were reverse transcribed and real time PCR were performed to quantify miRNAs expression. The results of miRNAs expression were given as fold change (fc) between HL-60.Bcr-Abl+ treated with TKIs and untreated HL-60.Bcr-Abl. Results: M downregulates miR-16 (fc=0.75), miR-21 (fc=0.55), miR-30c (fc=0.59) and miR-145 (fc=0.17) at four hours of NL treatment. CONCLUSION: TKIs are capable of modulating the microRNAs miR-16, miR-21, miR-30c, miR-145, miR-142-3p, miR-Ras and miR-15a which are involved in apoptosis, T cell proliferation and DNA repair machinery regulation in BCR-ABL+ cells. Thus, it seems that miRNAs expression profile may contribute to TKI response and suggest the potential of miRNAs as a new marker of CML prognosis.

1513
RELATIONSHIP BETWEEN MCV/MCH AND SEVERITY OF BETA GLOBIN GENE MUTATIONS IN B-THALASSEMIAS CARRIERS

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Thalassemia as a heterogeneous disease is one of the most common single gene disorder with a worldwide distribution. The aim of this study was finding a relationship between blood indexes and severity of beta globins gene mutations in beta-thalassemia carriers. In this cross-sectional study, we were determined 30 beta globin gene mutations in 1206 unrelated beta thalassemia carriers. Furthermore their blood indexes, including CBC and electrophoresis were also prepared. Then, by using SPSS software and t-test, the relationship between genetic findings and the results of their blood parameters were analyzed. In this study, the relationship between the severity of beta globin gene (β+ to β-), in beta thalassemia carriers, and their average blood indexes, were evaluated. The results indicated that β+ thalassemia in comparison with β0 thalassemia had a lower mean MCV and MCH value. That means with the less time and expense it could be possible to find a statistically significant relationship between a specific ranges of blood indexes and type of mutations in beta thalassemia carriers. The results confirmed a significant correlation with blood indexes and certain type of mutations in beta thalassemia carriers.

1514
HEMATOLOGIC DISORDERS CAUSING DIAGNOSTIC PROBLEMS IN DIABETES MELLITUS

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Aims: Investigation of diagnostic problems caused by hemoglobinopathies in diabetes mellitus, comparison of different chromatographic methods to determine glycated hemoglobin (HbA1c) in certain types of diabetic patients, following blood samples after in vitro glycation.

Methods: Glycated haemoglobin was determined by 5 chromatographic methods, including HPLC. The subjects were 2480 patients from Romania and 150 from Canada (including 27 persons with hemoglobinopathies). Statistical analysis of the experimental data was performed using the GraphPad InStat program. Results: The results of different chromatographic methods showed positive correlation (r=0.9, p<0.05), but elevated Hbf was found in 1.8% of the patients from Romania, and Hbf can be co-eluted with the HbA1c fraction in case of some chromatographic expression after four hours of treatment in HL-60.Bcr-Abl. In contrast, DAS upregulates miR-21 (fc=11.71) and miR-142-3p (fc=7.12). The levels of miR-let-7d decreased (fc=0.01) while let-7e (fc=2.89), miR-15a (fc=3.87) and miR145 (fc=11.97) increased in HL-60.Bcr-Abl after eight hours of NL treatment. CONCLUSION: TKIs are capable of modulating the microRNAs miR-16, miR-21, miR-30c, miR-145, miR-142-3p, miR-Ras and miR-15a which are involved in apoptosis, T cell proliferation and DNA repair machinery regulation in BCR-ABL+ cells. Thus, it seems that miRNAs expression profile may contribute to TKI response and suggest the potential of miRNAs as a new marker of CML prognosis.

1515
DERIVATIVE (6F17;16Q)(21P;P21): A RECURRENT CYTOGENETIC ABNORMALITY IN TWO CASES OF PEDIATRIC THERAPY-RELATED MYELODYSPLASTIC SYNDROME

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Childhood myelodysplastic syndromes (MDSs) are a rare group of clonal hematopoietic stem cell disorders occurring de novo or secondarily to cytotoxic chemotherapy and/or radiotherapy for a previous malignancy. Cyto genetic aberrations have been detected in about 50% of de novo MDS and in almost all therapy-related MDS (t-MDS) patients. The most common abnormalities are complete or partial loss of chromosome 7 and trisomy 8. Unbalanced rearrangements involving the long arm of chromosome 1 and leading to its trisomy, usually as t(1;7), are also recurrent. We report two cases of pediatric t-MDS with der(6)(1;6) (q21;p21). This unbalanced translocation has been reported in the literature associated with adult chronic myeloproliferative disorders. The first patient developed refractory cytopenia 9 years after suspension of chemo- and radiotherapy for a medulloblastoma diagnosed at the age of 7 months. The second patient, a 6-year-old boy, is slowly developing MDS features after a diagnosis of neuroblastoma at age 1 month and of anaplastic lymphoma 10 months later. Cyto genetic analysis of peripheral blood and bone marrow of the two patients revealed der(6)(1;6)(q21;p21). FISH for the painting of chromosomes 1 and 6 confirmed the rearrangement. Further array-CGH analysis performed on the bone marrow of the second patient, using 5500 BAC clones, showed gain of material of chromosome 1 (q21.1)[ARROWRIGHT]q44) and chromosome 6 (p22.1)[ARROWRIGHT]p12.1) and loss of material of chromosome 6 (p25.3)[ARROWRIGHT]p22.1). Array-CGH analysis is being carried out in the other patient to verify whether the imbalance is identical.

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CYTOGENETIC FINDINGS IN DE NOVO ACUTE MYELOID LEUKEMIA: A STUDY BASED ON 553 PATIENTS IN A SINGLE INSTITUTION OF GREECE

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Background. Acute myeloid leukemia (AML) is a heterogeneous disease with regard to clinical features and acquired genetic alterations. Currently, cytogenetic aberrations detected at diagnosis constitute the most common basis for predicting clinical outcome. Aims. We performed a conventional cytogenetic study of 553 de novo AML patients in order to define the chromosomal abnormalities and their frequencies as well as the frequencies of AML subtypes according to FAB classification. Methods: Chromosome studies were performed on unstimulated bone marrow cells, derived from 553 AML patients, aged ≥16 years at the time of diagnosis, between 2006 and 2010. Patients were classified according to FAB classification. Results. Three hundred ten patients were male and 243 were female. The median age of patients was 55.1 years (range 16-88). AML classification was available in 294 patients with M0 in 5.4%, M1 in 7.8%, M2 in 21.4%, M3 in 26.9%, M4 in 25.1%, M5 in 11.2%, M6 in 3.4% and M7 in 0.7% as unknown peaks on the chromatogram. Special attention is required in case of type 1 diabetic teenagers especially from rural areas, to prevent complications of the disease.
in 23%, -7 in 21%, -17 in 19%, del(5p) in 18.1%, -5 in 15.2%, -18 in 15.2%, del(7q) in 15.2%, -16 in 10.5%, -22 in 10.5%, -18 in 9.5%, -21 in 9.5%, -Y in 8.6%, t(8;21) in 7.6%, -20 in 6.7%, +20 in 6.7%, +21 in 6.7%, del(11q) in 5.7%, inv(16) in 5.7%, +11 in 4.8%, t(15;17) in 3.8% and chromosome-markers in 45.7%. Summary/Conclusions: AML with MDS/AML (sex ratio M/F was 28/98, median 68 years). FISH with locus probe Vysis LSI EGR1/D5S23, D5S721 Dual Color Probe (Abbott Molecular) and ON MDS 5q- (5q13, 5q35) / HETR (p518) TC (Kreatech Diagnostics) were done in all of them. To precisely determine the breakpoints, FISH with BAC clones (BlueGnome, 5q14.1-5q35.1 regions, multicolor banding (mBAND) for chromosome 5 (XCyte 5, MetaSystems) and array comparative genomics hybridization (aCGH, CytoChip Focus Haematology, BlueGnome) were performed. Results: In 119 patients (116 patients diagnosis 5q- was localized using both conventional CDRs, in four patients (8.0%) deletion of only distal CDR was confirmed. However, in three patients (2.5%) loss of the material on the long arm of chromosome 5 was outside of both CDRs - results of FISH with LSI probes for 5q31/5q35 regions did not prove the deletion. In patient No.1, a 61-year-old female with diagnosis AML, deletion 5q14q21 was confirmed in small clone (4%), out of 50 examined, whereas in the other 2 patients (25.5% and 15.2%) with deleted 5q, the abnormal karyotype was found in all examined mitoses. The same range of the deletion, del(5)(q14q25.3) was found in all examined mitoses. No. 2 and No. 3) with retained 5q31.2 and 5q32-q33.1 bands. To our knowledge, deletion of 5q14-5q22.2 as a sole aberration has not been described so far. Deleted region encompasses approximately 140 genes (http://www.ncbi.nlm.nih.gov/mapview/) and we assume that not only genes harbored in conventional CDRs but also genes localized in 5q14-5q22.2 region may contribute to malignant evolution of myeloid diseases.

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INTERPHASE FISH ON PURIFIED PLASMA CELLS IS SUPERIOR TO FISH ON CULTURED BONE MARROW CELLS IN PATIENTS WITH MULTIPLE MYELOMA

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Background. Multiple myeloma is a heterogeneous disease with a highly variable clinical course. Genetic abnormalities such as t(4;14)(p16;q32), t(11;14)(q13;q32), t(14;16)(q32;q23), 1p loss, 1q gain and hyperdiploidy have been shown to provide prognostic information (Fonseca et al. Leukemia, 2009). In multiple myeloma karyotyping is hampered by the low proliferative index of plasma cells and, therefore, in only about 30% of the patients an abnormal karyotype is found. Interphase fluorescence in situ hybridization (FISH) can circumvent this problem, but its resolving power is hampered by the overall low percentage of plasma cells present in bone marrow samples. Aim: To evaluate the diagnostic potential of FISH on purified plasma cells as compared to FISH on cultured bone marrow cells. Methods: We have developed and optimized a protocol for the purification of CD138+ cells (plasma cells) from bone marrow samples for FISH applications. Interphase FISH results obtained from such purified plasma cells were compared to those obtained from cultured bone marrow cells using an extended probe panel according to the recommendations of the Dutch Working group on Hemato-oncologic Genome Diagnostics (WHGD). This panel allows the detection of IGH rearrangements, including t(4;14),

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t(11;14) and t(14;16), 1p loss, 1q gain, 13q loss, 17p loss, and hyperdiploid status. A cytogenetic study on 21 patient samples showed that clonal genetic abnormalities can be detected in 98% of the patients when purified plasma cells were used. In 34% of these patients the genetic abnormalities were not identified when FISH was applied on cultured bone marrow cultures. Furthermore, FISH on purified plasma cells resulted in the identification of higher percentages of genetic abnormal cells, thus allowing the detection of genetic sub-clones. Summary/Conclusions. We conclude that interphase FISH on purified plasma cells superior to interphase FISH on cultured bone marrow cells for the detection of prognostic relevant genetic abnormalities in multiple myeloma.

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TRUE MONOSOMY OF CHROMOSOME 5 IS PRESUMABLY NOT AN ISOLATED CYTOGENETIC ENTITY IN MDS

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Background. Various aberrations of chromosome 5 are common cytogenetic findings in bone marrow cells of patients with MDS. The most frequent is interstitial deletion del(5q) occurring as sole chromosome abnormality or in combination with additional chromosomal changes. The size and breakpoints of deleted segment differs among patients, however the region 5q31 is commonly deleted in most of them. Two CDRs have been identified in 5q, indicating the presence of critical genes within those loci. Monosomy 5 is found by conventional cytogenetic methods in about 3-8% MDS cases only, usually in combination with other aberrations. Aims. The aim of this study was to perform detailed genome wide analysis in series of newly diagnosed patients with MDS and monosomy 5 detected by conventional cytogenetics, and to assess real frequency of true monosomy 5 in primary MDS. Methods: Fixed bone marrow cells of 46 MDS patients with suspected monosomy 5 found by G-banding (32 males, 14 females; median age 68 years) were retrospectively analyzed. To detect deletion 5q31 interphase FISH (I-FISH) with Vysis LSI EGR1/D5S23, D5S721 Dual Color probe (Abbott Molecular) was used. Complex aberrations were analyzed by mFISH/mBAND (MetaSystems). For identification of genomic imbalances BAC-based arrays (CytoChip Focus Hematology, BlueGnome) and/or SNP array (HumanCytoSNP-12 BeadChips, Illumina) were applied. Results: All 46 patients with suspected monosomy 5 presented a complex karyotype (≥3 chromosomal aberrations). Deletion of 5q31 was detected in 45 of them (97,8%) and loss of both regions (5p15 and 5q31) just in one by I-FISH. Whole genome molecular cytogenetic analyses confirmed that in all cases, parts of chromosome 5 material remains retained. No patient with true monosomy 5 was identified in this study. Common region conserved in all patients was established at 5p11.1-14.2 [22.31Mb]. mFISH/mBAND revealed cryptic translocations and insertions of chromosome 5 material to several chromosomal partners (chromosomes 17, 5, 7 and 12 as the most frequent ones). Finding of complex karyotypes involving deleted chromosome 5 at diagnosis was connected with poorer overall survival (median 3 months). Summary/Conclusions. In all patients with suspected monosomy 5, the parts of the deleted chromosome 5 have been shown to be retained elsewhere in the karyotype. Therefore, we believe that the true monosomy 5, quoted in the literature, in bone marrow cells of MDS patients probably does not exist. We assume that 5q deletion is arising in myeloid precursor cells as the primary event and subsequently leads to chromosomal instability and higher susceptibility to the rearrangements. This process is resulting in increased genomic damage and fast disease progression. In this study patients with deleted chromosome 5 involved into complex aberrations had an extremely poor prognosis.

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PATIENT-SPECIFIC MICRORNA EXPRESSION PROFILES IN CYTOGENETICALLY NORMAL ACUTE MYELOID LEUKEMIA AS A PLATFORM FOR MINIMAL RESIDUAL DISEASE DETECTION

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Background. MicroRNAs are a class of small noncoding RNAs playing a crucial role in the fine tuning of mRNA expression under physiological and pathological conditions. Recently, a number of reports demonstrated the applicability of genome-wide microRNA expression profiling in Acute myeloid leukemia (AML) for disease subclassification and prognostic stratification and suggested microRNAs as a novel clinically useful class of biomarkers. On the other hand, AML cases with normal cytogenetics (NC-AML) usually lack suitable molecular markers for minimal residual disease detection. It is, therefore, tempting to explore the utility of microRNA expression profiling for minimal residual disease detection. Aims. In this pilot study we aimed at examining the feasibility in clinical settings of identification of patient-specific profiles of overexpressed microRNAs in cases of NC-AML. Methods: We used a custom Flexmir v2 (Luminex Corp., USA) bead-based liquid assay for single-tube detection of 50 selected microRNAs. A total of 36 newly diagnosed cases of NC-AML cases were included in the study and 1 commercially available normal bone marrow RNA sample was included as a normalization control. Mean expression value for each microRNA in each sample was background corrected, normalized to the normal control, and log2 transformed. MicroRNAs were considered significantly overexpressed if the log2 value was ≥1.5. Results: Thirty (83.3%) cases had at least one significantly overexpressed microRNA. The number of overexpressed miRNAs varied between samples - 11 cases had 1-3 overexpressed microRNAs, 11 cases had 4-7 miRNAs and 8 cases had a profile with more than 8 overexpressed microRNAs. The most frequently overexpressed microRNAs were as follows: miR-19a (in 21 cases), miR-181a (in 20 cases), miR-19b (in 18 cases) and miR-17 (in 17 cases) whereas as a total of 15 microRNAs were not found to be overexpressed in either of the cases. Conclusions. We showed that it is possible to identify a patient-specific profile of overexpressed microRNAs in more than 80% of the cases of NC-AML cases. Besides, some of the overexpressed miRNAs were present in most of the cases, which makes it possible to use custom assays for just a few microRNAs (bead-based liquid assay, qPCR) for screening of overexpressed microRNAs in clinical settings.

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THE BIOINFORMATIC ANALYSIS OF ALU-REPEAT IN 5'-AREA OF MDR1 GENE PROMOTER REGION

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Alu repeats have obtained their name from the fact that most of them contain nucleotide AGCT, a cleavage site for the Alu I restriction endonuclease. A high concentration of Alu elements in the chromosome regions which contain a lot of genes allows duplication or elimination of genome fragments located between two Alu copies, as well as chromosome rearrangements. Alu repeats can affect the composition, organization and expression of the genome. Owing to their own promoter or enhancer activity, Alu repeats may enhance transcription of the adjacent locus. Transcriptional suppression is also possible, as Alu elements may expedite nucleosome assembly on the adjacent region. In addition, Alu repeats seem expedite methylation of neighboring loci, contributing to gene expression regulation. While methylation commonly suppresses transcription, cases are known when methylation of Alu repeats increases the transcriptional activity of the neighboring locus. Our aim was to investigate the interrupted Alu-repeat in 5'-area of multidrug-resistance gene (MDR1) promoter region. The investigation was performed on the DNA of lymphocyte cultures of healthy volunteers and on the DNA of leukemic cells of patients with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Totally 34 DNA samples (14 ALL, 10 AML and 10 of normal blood donors) obtained from mononu-

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Chimerism testing is a routine clinical decision measure of donor engraftment level in the recipient following allologeneic stem cell transplantation (SCT), with small percentage reductions used in the determination of therapy. Data from the last 5 samples issued (a total of 337 results) to 78 international laboratores participating in the UK NEQAS Post-SCT Chimerism External Quality Assessment programme were examined to determine if the number of Short Tandem Repeat (STR) markers used impacted upon the results returned. To allow data comparison the consensus median for each sample and the delta values from the relevant consensus median were calculated for each individual result. The larger the delta value, the further that data point was from the consensus median. Data was then grouped by the number of markers used in the calculation. Small reductions in the calculated delta values were observed when the methodologies were compared, (irrespective of the number of markers used in the calculation), in-house median delta was significantly larger than the commercial kits median delta (p=0.003). Statistical comparison of the number of markers used in the calculation by in-house compared to kits users is difficult as there is little overlap between the two sets of data (in-house users mode=2 markers compared to kit mode=8 markers). However, when comparing in-house users to kits users: on average they have smaller panels available (12 vs.14); analyse a smaller percentage of these analysed markers to be informative (63% vs. 68%); define a smaller percentage of these analysed markers to be informative (63% vs. 81%) and; use a smaller percentage of these informative markers in their final donor calculation (60% vs. 68%). In conclusion, our data has shown that statistically, results generated by in-house methodology have a larger delta value (further from the median) than those generated by a commercial kit. This difference may be due, in part, to the number of markers used in the calculation. However, this study has highlighted the urgent need for guidance and standardization in clinically significant post-SCT chimerism testing.

Deletions of the long arm of chromosome 6 (6q) are known to occur at relatively low frequency (3-6%) in B cell chronic lymphocytic leukemia (B-CLL), and are more frequently observed at 6q21. Patients with B-CLL carrying a 6q21 deletion show clinicobiologic features, and their outcome should be allocated in an intermediate-risk category. Few data have been reported about other band at 6q with cytogenetic alterations in B-CLL. In the current study, we used Bacterial Artificial Chromosome (BAC) clones as probes for fluorescence in situ hybridization (FISH) to analyze the incidence and localization of 6q aberrations in 110 cases of B-CLL at diagnosis. In this study, we have four BAC clones mapping regions in bands 6q16 (RP11131A17), 6q23.3 (RP11323N12), 6q25.2 (RP11559G2), 6q27 (RP1137D8). The FISH study was performed on nuclei and metaphases after stimulation with a combination of CpG-oligonucleotide DSF50 and interleukin 2 (IL-2) as previously described. Of 110 samples studied with our set of BAC clones probes, 94% could be successfully analyzed by interphase-metaphase FISH. We identified 11 cases (10%) with 6q deletion, the percentage of cells containing 6q deletions ranged from 7% to 89%. Both trisomy for chromosome 12 and homozygous deletion of 13q14 as well as no anomaly were found in 4 cases (3.6%). In 6q16 using FISH, 6q25.2 deletion in 6q16 using FISH. Trisomy for chromosome 12 associated with homozygous deletion of 13q and loss of 17p were demonstrated in 1 patient with a 6q23.3 and a 6q25.2 deletion using FISH. We also observed a 6q25.2 deletion without other aberrations in 2 cases using FISH. In one patient was found deletion by our set of BAC clones probes in all investigated bands associated with 6q23 deletion in FISH. One case showed deletion of 6q16 and 6q25.2 coupled with trisomy 12. In one sample 6q27 deletion was associated with homozygous deletion of 13q and loss of 17p13. The last case delet ed in 6q25.2 and 6q27 showed no other anomalies in FISH. In our study, of 11 cases with 6q deletion, 6 have the data of chromosome banding analysis (CBA) available. In 4 cases the abnormalities observed in addition to the 6q deletion were a trisomy 12, a loss of 13q, 17p, 14q, a gain of 7p or presence of marker chromosome. In addition 2 cases with deletion 6q show a complex aberrant karyotype. Moreover six results showed borderline value which should be further investigated. Previous cytogenetic studies suggest that tumor suppressor genes that are involved in the pathogenesis of malignant lymphoid diseases have been found at 6q. In this study, using a panel of BAC clones probes, we detected deletion in the region 6q16, 6q23.3, 6q25.2 and 6q27, and we identified 11 cases with a 6q deletion, but only one showed a deletion of at 6q21, which is recognized by the commercially available probe. We are correlating cytogenetic data obtained with clinical course to evaluate the prognostic value of 6q deletions. Our data revealed that the 6q deletion is characterized by increased genomic instability and suggest ed that these deletions represent a secondary event in tumorigenesis of CLL.
DOES ADDITIONAL ABERRATIONS DETECTED BY METAPHASE CYTOGENETICS \textit{vs} FISH analysis be a relevant tool in the treatment and monitoring of \textit{cML} patients? This question was addressed in a study by E Wawrzyniak, A Kotkowska, J Blonski, T Robak, and their colleagues. The aim of the study was to investigate the use of additional methods for detecting additional aberrations in \textit{cML} patients. The study compared the results of different methodologies and aimed to assess their accuracy and reproducibility.

**Background**: The study was conducted in a hospital in Poland, focusing on patients with \textit{cML}. The researchers used various methods to detect additional aberrations, including metaphase cytogenetics, FISH analysis, and qPCR. The study included patients with single or multiple additional aberrations, and the results were compared to the standard methods used in clinical practice.

**Methods**: The study included a total of 52 patients with \textit{cML} who were divided into two groups based on the presence of additional aberrations. Group A included patients with additional aberrations detected by metaphase cytogenetics, whereas group B included patients with additional aberrations detected by FISH analysis. The researchers performed a comparative study on clinical samples with known percentages of tumour cells expressing different fusion transcripts. They also performed a comparison of the results obtained using different methodologies.

**Results**: The study showed that the accuracy of detecting additional aberrations using FISH analysis was comparable to that of metaphase cytogenetics. However, FISH analysis provided additional information about the nature and location of the aberrations, which was not available from metaphase cytogenetics. The researchers also found that the reproducibility of FISH analysis was higher than that of metaphase cytogenetics, which could be due to differences in the methodology and the complexity of the samples.

**Conclusions**: The study concluded that FISH analysis could be a valuable tool in the treatment and monitoring of \textit{cML} patients, especially when initial results are inconclusive. The researchers suggested that FISH analysis should be considered as an alternative method to metaphase cytogenetics for detecting additional aberrations in \textit{cML} patients. The study also highlighted the need for further research to improve the accuracy and reproducibility of FISH analysis.
translocated to der(21). Metaphase FISH using RP11-838G2 labeled SpectrumOrange, HOXA gene flanking probe on telomere side, and RP11-1025C19 labeled SpectrumGreen, HOXA7-13 spanning probe showed one fusion signal on normal chromosome 7, one small fusion signal on der(21) and small green signal on der(7). This finding indicates that the region including HOXA7-13 or part of HOXA7-13 is translocated on der(21). Conclusions. These results clearly demonstrate that the translocation of RUNX1 and HOXA gene was involved in acute myeloid leukemia and suggest that juxtapositioning of the RUNX1 and HOXA genes would be a novel mechanism for leukaemogenesis.

1528 EVALUATION OF A QUALITATIVE IN VITRO DIAGNOSTIC DEVICE FOR THE MULTIPLEX DETECTION OF SPECIFIC FUSION TRANSCRIPTS TO AID IN THE DIAGNOSIS OF LEUKEMIA

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Background: Multiple recurring chromosomal translocations occur in leukaemia. Sensitive detection and accurate classification of the associated gene fusions is critical for the diagnosis and prognosis of many leukaemias. At the molecular level the breakpoints can vary over a wide region within the genes involved, and it is often necessary to determine the specific fusion sub-type for the purposes of ongoing monitoring with quantitative molecular techniques. Aims: To evaluate a new commercially available CE-marked IVD test kit in a clinical setting for the simultaneous detection of leukaemia fusion transcripts in a single multiplex assay. To determine diagnostic specificity and sensitivity of the test relative to standard cytogenetic and molecular methods. Methods: Archived total RNA samples from leukaemia patients at presentation were analysed using the commercial kit. The test can detect 12 fusion transcripts (BCR-ABL1 e1a2, BCR-ABL1 e1a3, BCR-ABL1 e1a2, BCR-ABL1 e1a2, TCFS5-PBX1, ETV6-RUNX1, MLL-AF4 e9e5, MLL-AF4 e10e4, PML-RARA bcf1, PML-RARA bcf1, CBFB-MYH11 type A, CBFB-MYH11 type D, RUNX1-RUNX1T1) and includes GAPDH as an endogenous control to assess sample quality. Following multiplex RT-PCR using 100 to 400 ng RNA per test, the identity of biotin-labelled PCR products was simultaneously determined by hybridization on a liquid bead array, containing target-specific probes, and flow cytometric detection. Qualitative calls (positive or negative for each target) relative to a fixed cut off signal (350 MFI) were generated and compared to known diagnostic status. Results: To date, 70 specimens have been evaluated, 58 positive and 12 negative. One sample failed (no signal) and one positive sample generated a low signal at 294 MFI, below the fixed cut off signal (350 MFI). These 2 samples are being further investigated. For the 69 samples with results, a total of 759 calls were generated for the individual fusion transcripts corresponding to an overall agreement with reference methods of 99.9% (758/759; 95%; CI: 99.3 to 100%). Diagnostic sensitivity was 98.2% (56/57; 95% CI: 90.7 to 99.7%) and diagnostic specificity was 100% (702/702; 95% CI: 99.5 to 100%). Summary/Conclusions: The assay is quick, simple and reliable. It detected all twelve fusion transcript types in representative clinical specimens. Analytical sensitivity was not evaluated here (1% reportable). It has the advantage of typing individual fusions, and assigned these correctly in 100% of cases. This could facilitate the downstream use of specific molecular assays such as RT-qPCR for follow-up analyses and minimally invasive monitoring. Finally, multiplex detection as a single reaction can greatly improve laboratory efficiency, which would be further enhanced by the addition of more rare fusion subtypes such as BCR-ABL1 e1a3e3 and e1a3e4 or CBFB-MYH11 type E to the kit. Overall the CE-marked IVD test kit is an attractive novel molecular method that is compatible with the clinical laboratory workflow and complements standard cytogenetic methods.

1529 SMALL B-CELL LYMPHOPROLIFERATIVE DISORDERS DISPLAYING CLL LIKE FEATURES AND HARBOURING TRISOMY 3

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Trisomy 3 was first reported as a sole abnormality in adult T cell lymphoma/leukaemia and subsequently in other subtypes of T-non Hodgkin lymphoma (NHL), particularly lymphoepithelioid and angioimmunoblastic. Later, it was identified as the most frequent abnormality in marginal zone B-cell lymphoma. In other B cell NHL it is relatively rare and is reported only sporadically in chronic lymphoproliferative disorders (CLPD). Although it is not a recognised recurrent abnormality associated with chronic lymphocytic leukaemia (CLL), it is detected in 1.5%-2.8% of karyotypically abnormal cases and in around 8% of all cases by FISH. Given the importance of recognising new cytogenetic entities with differing clinical course and prognosis we set out to determine whether trisomy 3 is a rare recurrent abnormality associated with CLL or whether the presence of this abnormality in the karyotype should evoke a differential diagnosis. Twelve cases with a trisomy 3, complete or partial, for whom a diagnosis of CLL was retained by the referring institution, were retrieved from the databases of two Belgian genetic centres. The sam- ple size was 13 cases. In four cases, both bone and blood and bone marrow in one. The clinical, biological and histological findings of these presumptive cases were reviewed. A diagnosis of typical CLL, or atypical-CLL, was retained for cases with either a Maturet- Cakovsky score of ≤2 or histological confirmation of CLL on review. Five were associated with Binet stage A and two with Binet stage B disease. On follow up, three had stable disease, two showed evolutive disease requiring treatment and two underwent Richter transformation. Morphological examination of all cases was consistent with CLL. Three cases displayed a typical immunophenotype with a score of 4 or 5, three had a score of 5 and one a score of 2, included after histological confirma- tion of typical CLL. One had a raised monoclonal IgM and another cold agglutins. Trisomy 3 was present as the sole abnormality in 3 cases, was associated with one other aberration in 2 cases (balanced translocation, trisomy 12), and occurred as part of a complex karyotype in 2 cases (>5 abnormalities). Interestingly, the two cases that under- went Richter transformation had a complex karyotype. To date, the clinical details associated with trisomy 3 have only been evaluated in 3 cas- es of CLL (Wong 2002, Specchia 2002, Michaux 1998). For these cases a diagnosis of CLL was based on morphological criteria despite an atypi- cal phenotype. Unlike those previously reported three of our cases had a typical CLL immunophenotype. This study brings the total number of reported cases of trisomy 3 associated CLL to twelve. Morphological criteria was consistent with CLL in all cases. Phenotype was frequently atypical, but not evocative of any other specific disease entity. Whether these atypical cases represent true CLL or a distinct subtype of CLPD with CLL like features remains to be determined. Indeed, as suggested by others, these cases may in the future correspond speculatively to a new disease entity warranting further investigation.

1530 ANTICIPATION IN FAMILIAL HEMATOLOGICAL MALIGNANCIES

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Background. We observed thirty-four families with at least two members who presented a hematological malignancy. Whereas there are a lot of studies about it on solid neoplasms, there is not a good knowledge of it in the hematological field. Aims. Our purpose was to evaluate the anticipation in our experience. Methods. We observed thirty-four families with at least two members who presented a hematological malignancy diagnosed from 1950 to 2010. The male-to-female ratio was 42:58; they were affected by Non-Hodgkin’s Lymphoma (25 pts), Chronic Lymphocytic Leukemia (11 pts), Hodgkin’s Lymphoma (5 pts), Acute Myeloid Leukemia (8 pts), Chronic Myeloid Leukemia (4 pts), Myeloproliferative Neoplasms (4 pts), Multiple Myeloma (4 pts), Acute Lymphoid Leukemia (2 pts), Waldenstrom’s Macroglobulinemia (1 pt) and Acute Leukemia non otherwise specified (6 pts). At this time 30 patients were dead. Two main different generations were involved in twenty-two families including forty-nine patients. We have restricted our analysis to two generations because of the small number of cases in the previous ones. Then we studied fourteen families in which two or more
members are affected by hematologic malignancies (HMs) among the same generation. Anticipation is assessed with a logrank test for trend using GraphPad Prism software version 5.0. Results. The median age at diagnosis for patients of the first generation was 58 years (range 9-82 years) whereas the median age at diagnosis for patients of the second one was 30.5 years (range 3-61 years).

We demonstrated the phenomenon of anticipation between the second and the first generation with an important and significant difference of 28.5 years (p=0.0003) (Image). The analysis on patients of the same generation showed that the median age at diagnosis for first-borns was 61 years (range 23-75 years) and for the second-borns was 55 years (range 17-68 years), with a difference of 8.0 years, which was not statistically significant (p=0.249). Conclusions. It is clearly recognized in the literature that there is a genetic basis for familial risk of HMs; however the causative mutation has not been identified. Future studies on these families should help to clarify genetic pathways underlying familial HMs.

1531 USEFULNESS OF IGH/TCR CLONALITY PCR STUDIES IN LYMPHOPROLIFERATIVE DISORDERS WITH DOUBTFUL CLONALITY BY FLOW CYTOMETRY
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Background. The kappa/lambda index in flow cytometry (FCM) is used to check clonality in B-cell diseases. A clonal result is considered when this index is ≥3 or <0.5. However, in borderline results it is difficult to assess the clonality. For T-cell clonality assessment the qualitative status of CD expression (crosslineage or asyncronous expression) and/or the quantitative status (absolute number of T cells or CD4/CD8 ratio) is used in FCM. Aims. To assess the clonality by PCR in patients with lymphoproliferative disorders with doubtful clonality by FCM. Samples DNA was extracted from 92 samples of peripheral blood, bone marrow, lymph node aspirates or biologic fluids from patients with suspicious lymphoproliferative disorders in whom the clonality assessment by FCM was doubtful.

Three different regions of the IGH VDJ and TCRγ VDJ segment were amplified by PCR using a commercial kit (Invivoscribe Technologies, San Diego, USA) and analysed by Genescan. A study was considered polyclonal when a Gaussian distribution of transcripts was obtained and clonal when the area of the highest peak was three times bigger than the area of the third highest peak. A doubtful result was a peak 2-3 times higher than the polyclonal population without reaching the limit of clonality. A non-informative result was given when no IGH or TCRγ amplification was observed but the specimen controls were correct. Results. Table 1 shows the frequency of informative and non-informative PCR results, as well as the correlation with clinical diagnosis for samples evaluated at diagnosis. Summary. PCR was informative in 76% of samples from patients with lymphoproliferative disorders with doubtful clonality by FCM, of whom 39% were clonal. Hematogenous neoplasm was confirmed in 56% of clonal disorders assessed by PCR at diagnosis.

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1532 MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) A SENSITIVE TECHNIQUE FOR THE DETECTION OF TP53 DELETIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA?
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Background. Detection of a deletion of TP53 on chromosome 17p13 influences choice of treatment in previously untreated or relapsed patients with CLL. Fluorescence in-situ hybridisation (FISH) is widely used for the detection of TP53 loss however the gold standard, if there is one, for the diagnosis of TP53 loss in CLL has never been published. Multiplex Ligation-dependent Probe Amplification (MLPA), a molecular technique able to simultaneously detect copy number aberrations (CNA) of multiple loci in a single PCR reaction, has been reported as a non-subjective, accurate, cost-effective and high-throughput alternative technique for the detection of CNA in CLL. Although the number of reported cases with deletions of TP53 are too few to reliably assess the efficacy of this technique in the detection of this important gene deletion. The aim of this study was to investigate the specificity and sensitivity of MLPA for detecting known deletions of TP53 in CLL. Methods MLPA analysis was performed on peripheral blood mononuclear cell DNA using two commercial kits (P037 and P038, MRC Holland) designed to detect recurrent CNA in CLL. Each kit contains four different probes targeting TP53. The normal range for each individual MLPA probe was established from 36 normal control samples and the mean ±2SD was used to determine the cut off limits for gain or loss. Blinded analysis was performed with one or both kits on a total of 64 CLL samples previously screened by FISH with Vysis/Abbott LSI TP53/CEP 17 probes. Of the 64 samples, 44 had TP53 deletion in 5-96% (median=76 %) of cells and 20 had no detectable TP53 deletion by FISH. Samples were categorised as TP53 deleted by MLPA when ≥2/4 of the TP53 probes in a single kit, or ≥3/8 TP53 probes across the two kits, were below their defined cut off limit. Results. The sensitivity of MLPA for correct identification of samples showing TP53 deletion by FISH was 90% (37/41, 95ci=79-97%), 76% (28/37, 95ci=61-87%) and 88% (29/33, 95ci=74-96%) for the P037, the P038 kit and combined kits, respectively. The P037, P038 and combined kits all showed 100% specificity (20/20, 95ci=87-100%) for correct identification of samples without TP53 deletion by FISH. All four samples with TP53 deletion detectable by FISH but not by the P037 kit alone, or in combination with the P038 kit, had deletion in ≥14% of cells. Looking across the control sample data, the standard deviations for probes from the P038 kit were significantly higher than those from the P037 kit (p=0.01), leading to a wider normal range, possibly accounting for the lower sensitivity of the P038 kit. Conclusions. TP53 deletions in CLL samples can be reliably detected by MLPA using the combined data from the P037 and P038 MLPA kits provided deletion is present in approximately 20% or more of the tumour cells. This is close to the clinically relevant clone size as determined by FISH and as such MLPA shows potential for adoption in a routine setting subject to further validation.

1533 STUDIES ON COMMON SPLICE VARIANTS OF PROGNOSTICALLY IMPORTANT FUSION ONCOCGENSES IN PAKISTANI LEUKEMIA PATIENTS: IMPLICATION IN LEUKEMIA BIOLOGY, DIFFERENTIAL DIAGNOSIS, PROGNOSIS AND TREATMENT
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Background. Three different regions of the IGH VDJ and TCRγ VDJ segment were amplified by PCR using a commercial kit (Invivoscribe Technologies, San Diego, USA) and analysed by Genescan. A study was considered polyclonal when a Gaussian distribution of transcripts was obtained and clonal when the area of the highest peak was three times bigger than the area of the third highest peak. A doubtful result was a peak 2-3 times higher than the polyclonal population without reaching the limit of clonality. A non-informative result was given when no IGH or TCRγ amplification was observed but the specimen controls were correct. Results. Table 1 shows the frequency of informative and non-informative PCR results, as well as the correlation with clinical diagnosis for samples evaluated at diagnosis. Summary. PCR was informative in 76% of samples from patients with lymphoproliferative disorders with doubtful clonality by FCM, of whom 39% were clonal. Hematogenous neoplasm was confirmed in 56% of clonal disorders assessed by PCR at diagnosis.

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1) E2A-PBX1 (t 1;19), MLL-AF4 (t 4;11) are higher in Pakistani pediatric ALL patients than western counterparts (74.2% vs 27%, p=0.01) while reverse is the case of TEL-AML (16.5% vs 25%, p=0.01) associated with significant difference between Leukemia patients from Pakistan and adult AML patients in Pakistan(7). Our studies have implications in clinical management of Leukemia at healthcare policy-making bodies and adult AML patients in Pakistan(7).

4) Gene frequencies, which is in accordance with other reports(5,6) which may be related to ethnicity. These results explain the molecular genetic basis of already-reported poor prognosis and survival of paediatric ALL and adult AML patients in Pakistan(7). Our studies have implications in clinical management of Leukemia at healthcare policy-making bodies and clinical centers.

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**1534 RARE CYTOGENETIC FEATURES IN ACUTE PROMYELOCYTIC LEUKEMIA**

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Acute promyelocytic leukemia (APL) is strongly associated with t(15;17)(q22;q21) giving rise to the formation of two functional fusion genes, PML/RARA on the derivative chromosome 15 and RARA-PML on the derivative chromosome 17. A small group of patients with APL appears to have a submicroscopic insertion of the RARA gene into chromosome 15 or microscopically visible rearranged rearrangement of derivative 17. The prognostic significance of these genetic abnormalities is still under discussion. Among 13 newly diagnosed acute myeloid leukemia patients in 2010, 3 (23%) were classified as an acute promyelocytic leukemia with t(15;17) according to the WHO 2008 criterias. Typical t(15;17) was recognised by classical cytogenetic analysis in one case (case1, F/61y.o). Hematologic workup showed t-AML after previously treated breast cancer with PML-RARA fusion gene transcripts, FLT3-ITD and WT1 mutation. She was in minimal residual disease after 1th induction chemotherapy with cytarabin, idarubicin and ATRA. In the second patient (case1, M/27y.o), chromosome analysis showed a banding pattern compatible with an insertion (17;15) or *two way transloca-
**1535 MUTATIONS IN IDH1/2 GENES IN ACUTE MYELOID LEUKEMIA WITH NORMAL KARYOTYPE: A SINGLE CENTRE STUDY OF 72 CASES**

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**Background.** Normal karyotype acute myeloid leukemia (NK-AML) comprises a clinically and biologically heterogeneous group of AMLs accounting approximately for 45% of all cases. A number of molecular changes including mutations in FMS-like tyrosine kinase 3 (FLT3), nucleophosmin (NPM1) and CCAAT/enhancer binding protein alpha (CEBPA) genes provide either alone or in combination prognostic information particularly in NK-AML. Recent mutations in genes for the isocitrate dehydrogenase 1 and 2 (IDH1/2) have been reported recurrently in AML. The prognostic impact of these mutations still remains to be determined however their potential as candidate markers for monitoring minimal residual disease is of special interest in NK-AML. **Aims:** In this study we investigated the prevalence of IDH1/2 mutations in a cohort of NK-AMLs. We aimed to correlate IDH1/2 mutation status with clinical features, prognostic impact and additional recurrent mutations in AML occurring in FLT3, NPM1, CEBPA, WT1 and MLL genes using fragment analysis and/or direct sequencing. An allele specific polymerase chain reaction assay with a sensitivity of ~1% was introduced to detect IDH1, R152H mutation in post-treatment samples. Results: An earlier study of 25 NK-AMLs were found to carry heterozygous IDH1/2 mutation with IDH1 mutations detected in 7 patients (10%, R132H only) and IDH2 mutations in 13 patients (18%; 12 cases with R140Q and 1 case with R172K). We revealed 9 patients (13%) with a synonymous IDH2 mutation with IDH1 mutations detected in 7 patients (10%, R132H and 12% with R140Q and 1 case with R172K). We revealed 9 patients (13%) with a synonymous (GCC/GGT) IDH1 single nucleotide polymorphism rs1154197. Additionally one patient presented with so far not identified T100M (ATG ATC) IDH1 sequence variants. In our cohort IDH1 and IDH2 mutations were mutually exclusive. Both IDH1 gene mutations tended to coexist with NPM1 (11/20) and/or FLT3 (8/20) mutations. However in only 2 cases we found IDH2 R140Q mutation accompanied with concurrent mutation in CEBPA or MLL genes respectively. IDH1/2 mutation status did not correlate significantly with clinical characteristics of patients regarding age, sex, leukocyte count, bone marrow or peripheral blood blast counts. The differences were not statistically significant when comparing overall and disease-free survival between the IDH1 mutated and wild-type groups. **Summary/Conclusions:** Our data provide evidence on reduced IDH1/2 mutations that are mutually exclusive in our cohort of NK-AML and tend to overlap with prognostic mutations such as FLT3 and/or NPM1. Further evaluation of IDH1/2 mutations in post-treatment samples is in process and will determine the usefulness of these mutations in monitoring minimal residual disease in AML. **Funding.** Supported by MSM 619895205 and LF-2011-006.
It has been recently reported the activity of 4-aryl-4H-chromenes family, to induce apoptosis in human cancer cells. Herein we report a derivative of 4-aryl-4H-chromene compound with higher apoptotic activity against Erythroleukemia K562. The cells were seeded in 24-well plates at 1x10^5 cells/well and treated with 50-80 nM of the 2-amino-4-(3-nitro phenyl)-3-cyano-7-(dimethylamino)-4Hchromene (2-NC). This compound was found to be highly active growth inhibitor with IC50 of 65±3.5 nM as determined by MTT assay. Proliferation of K562 cells was diminished by more than 70% and viability was decreased by about 50% upon 72 h treatment with 50-80 nM concentration of the compound. Apoptosis as the mechanism of cell death was investigated morphologically by Hoechst 35248 staining, caspase-3 activation assay, as well as the formation of DNA ladder. K562 cells underwent apoptosis upon a single dose (IC50 value) of the compound and also increased caspase-3 activity by more than 2-fold following 72 h treatment. Caspase-9 was also activated which could be detected 48 hours post-treatment. Furthermore, Western blot analysis revealed that the treatment with the compound down-regulated the expression of certain IAP protein including survivin. Considering the negative effects of survivin on caspase activity, one can propose that the treatment of cells with 2-amino-4-(3-nitro phenyl)-3-cyano-7-(dimethylamino)-4Hchromene leads to degradation of survivin protein unleashing caspase activity and causing apoptotic cell death. Given the fact that survivin confer resistance to certain cancer cells and considering apoptotic cell death of cancer cells due to activation of caspases upon degradation of survivin, one can propose the down regulation of survivin as an effective approach for cancer therapy under conditions in which these proteins confer resistance to cancer therapy. These data further suggest that 2-amino-4-(3-nitro phenyl)-3-cyano-7-(dimethylamino)-4Hchromene (2-NC) may provide a novel therapeutic approach for the treatment of leukemia.
METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) POLYMORPHISMS IN PATIENTS TREATED WITH INTERMEDIATE-HIGH DOSE OF METHOTREXATE (MTX)

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Background: Association with MTHFR polymorphisms and toxicity or outcome of disease was investigated in haematological patients treated with methotrexate, with no univocal results. Methods: We retrospectively studied 22 patients (14 M / 8 F), median age 40 years, (range 27-63), affected by Non Hodgkin lymphoma (3 Mantle cells, 9 burkitt, 6 DLBC with S CNS localization, 1 anaplastic T cells, and 1 Acute Lymphoblastic Leukemia, submitted to different chemotherapeutic regimens, all containing intermediate/high dose of MTX for a total of 61 courses. We divided patients in two groups: group A submitted to intermediate dose of MTX (1-2g/m2), group B with high dose of MTX (3-5g/m2). We considered in particular hepatic toxicity and incidence of mucositis, in association with delayed elimination of MTX. All patients were tested for MTHFR polymorphisms C677T and A1298C. Results: Group A: 9 patients, for a total of 25 courses. In 6/25 courses (24%), we have a delay in MTX elimination (>72h); hepatic toxicity of grade II-III were recorded in 7/25 (28%), mucositis in 16/25 (64%), 12 of grade I, 3 of grade II, 1 of grade III. Two patients showed a very high delay in MTX elimination (>120 hours), with mucositis of grade I/II without hepatic toxicity. Among 9 patients, 7 were mutated for C677T (5 heterozygosis, 2 homozygosis) and 2 were not mutated for both polymorphisms. The two patients with higher toxicity were not mutated. Group B: 14 patients, for a total of 36 courses. A delay of MTX elimination was seen in 20/36 (55%), hepatic toxicity in 18/36 (50%), 2 of grade III, 7 of grade II, 9 of grade I, mucositis in 21/36 (59%), 11 of grade 1, 7 of grade II, 3 of grade III. In 9 out of 20 delayed MTX courses we recorded a very high delay (>120h) with hepatic toxicity in 6/9 and mucositis in 9/9 courses. Among these 14 patients, 3 showed C677T heterozygosity, 2 C677T homozygosity, 3 showed A1298C homozygosity and 6 showed both mutations in heterozygosity. In 12 out of 14 patients grade III-IV of haematological toxicity was seen, among the 12 patients 8 were treated with an association with ARA-C, 3 were treated with polichemotherapy, while one patient received only MTX. Conclusions: We were not able to find any correlation between toxicity and MTHFR polymorphisms in this subset of patients.

HLA ALLELES AND HAPLOTYPES: IMPLICATIONS FOR HEMATOPOIETIC STEM CELL DONOR SELECTION

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Background: Despite expansion of unrelated donor pools to a current state of more than 10 million registered donors worldwide, matching each HLA allele between unrelated donors and recipients remains a problem for many patients because of HLA polymorphism. Consideration of other related donor available is appropriate for any patient who is in need of an allogeneic hematopoietic cell transplant but has no matching sibling or unrelated donor or cord blood transplant. Analysis of common alleles and haplotypes has the potential to predict the chance of finding a suitable donor, and it also suggests strategies for improving success in the search. Aims: Our objective is to establish which HLA alleles and haplotypes are most common in our area. These results can be used to help in delineate search strategies for potential donors: related or unrelated Subjects and methods: The study includes 200 families residing in Andalucía, Spain. Informed consent was obtained in all cases. They were HLA A∗, B∗, C∗ y DRB1∗ typed by high-resolution (Dynal RELITM SSO typing Kit, Dynal Biotech ASA, Oslo and Inno-Lipa HLA, Innogenetics NV. Ghent, Belgium). Results: Only 35 HLA-A∗, 55 HLA-B∗, 23 HLA-C∗ and 39 HLA-DRB1 alleles were found. These alleles have been previously described in Caucasians as expected from the geographic origin of the studied families. The distribution of predominant alleles studied herein reveals great similarities with those in Spain, Western European and North American populations. In the analysis of haplotypes, their frequencies in the studied population were stimated by counting the number of any given haplotype among the total number of haplotypes. A high number were included within the most frequent haplotypes described in Spain. However, the results reveal that the ten most frequent haplotypes in our population were not the same as in other populations Summary: The data we present here on common alleles and haplotypes may help to predict the chance of getting a suitable related donor, and it also suggests strategies for improving success in the search.

LIPOPROTEIN PROFILE IN IRON DEFICIENCY ANEMIA

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Background: Subjects and methods: A total of 20 patients (males/females: 7/13, mean age 41±15 years) with IDA were retrospectively examined. They were eligible for participation if they met the following criteria: Hb<12 gr/dl, MCHC<32%, RDW-CV>15%, serum ferrum <37 μg/dl, serum ferritin <15 μg/L. 20 age and sex-matched healthy subjects were also included. Methods: Serum iron parameters (ferrum, ferritin) were determined by electrochemiluminescence immunoassay, lipid parameters (total cholesterol, TC, high density lipoprotein, HDL, triglycerides, TG) with enzymatic colorimetric test (Elecsys Modular E170, ROCHE) and hematological indices (hemoglobin, HB, hematocrit, Ht, mean corpuscular volume, MCV, mean corpuscular haemoglobin, MCH, mean corpuscular hemoglobin concentration, MCHC) by cytometry method (XE-5000 SYMEX, ROCHE). Student’s t test and Pearson’s correlation analysis were performed. Results: In patients with IDA, TG levels were found to be significantly elevated (101.30±53.07 vs 95.56±42.43 mg/dl, p<0.05) and HDL-C concentrations significantly lower (49.1±12.92 vs 56.62±19.19 mg/dl, p<0.05) when compared to controls. TG/HDL-C ratio, which is considered indicative of the predominance of small, dense LDL particles, was found higher in IDA subjects (2.17 vs 1.70, p<0.05). No significant

PHARMACOGENETICS OF COUMARINS DOING: PREVALENCE OF CYP2C9 AND VKORC1 POLYMORPHISMS IN THE LEBANESE POPULATION

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Background: Polymorphisms in the genes encoding the cytochrome P450 2C9 enzyme (CYPC29) and the Vitamin K Epoxide Reductase (VKORC1) are known to contribute to variability in sensitivity to coumarins. Patients with certain common genetic variants of CYP2C9 (*2 & *3) or a VKORC1 polymorphism (-1639A Allele) require a lower dose of coumarin and are also at higher risk for over-anticoagulation and serious bleeding. In August 2007, the FDA label for warfarin was updated to highlight the benefit of genetic testing to predict warfarin response. Aims: Since the prevalence of these variants in the Lebanese population has not yet been reported, our aim was to determine the genotypes of CYP2C9 and VKORC1 in our population and to compare allele frequencies with previous findings from other ethnic groups. Methods : CYP2C9 (*1/*2/*3) and VKORC1 (*A/*G) allelic variants were assessed by Polymerase Chain Reaction-Restriction Length Polymorphism (PCR-RFLP) assays in a diversified sample of 161 unrelated healthy Lebanese volunteers. Results: The allele frequencies of CYP2C9 *2 and *3 were 0.112 and 0.096 respectively, whereas VKORC1 -1639A Allele represented 34.2% of the subjects, while those of the VKORC1 -1639A represented 73.9%. Conclusion: Our data show no significant difference in the frequency of CYP2C9 allelic variants when compared to the Caucasian population, whereas the allelic frequency of VKORC1 -1639A was very high. Over 50% of the Lebanese population seem to be carrying more than two independent “risk” alleles, and is therefore potentially at high risk of over-anticoagulation.
differences were observed in TC and LDL-C concentrations as well as in TC/HDL-C ratio between patients and controls. HT and Hb values presented a significant inverse correlation with those of TG (r = 0.85, p = 0.031 and r = -0.34, p = 0.039 respectively) and a significant positive one with those of HDL-C (r = 0.386, p = 0.023 and r = 0.420, p = 0.011 respectively). Summary/Conclusions. The findings suggest that lipid profile changes are preserved when serum iron and ferritin levels are decreased. The effect of IDA and its degree on atherosclerosis should be further investigated.

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IN VITRO DETERMINATION OF APOPTOTIC EFFECT OF HEPARIN ON LYMPHOCYTES BY USING FLOW CYTOMETRIC DNA ANALYSIS AND MEASUREMENTS OF CASPASE-9 ACTIVATION AND CYTOCHROME C LEVEL
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Background: Heparin induces apoptosis on peripheral neutrophils, mononuclear cells of healthy subjects and on lymphoblasts of patients with acute lymphoblastic leukemia (ALL), in vitro. Aims: Caspase-9 activity and cytochrome C level were studied to indicate the apoptotic effect of heparin on lymphoblasts by intrinsic pathway of apoptosis. Methods: Twenty bone marrow samples of the patients with ALL were included in the study. Cytochrome C level and caspase-9 activity were concomitantly determined with the percentage of apoptotic lymphoblasts when incubated in 0, 10 and 20 U/ml heparin concentrations at 0.1 and 2 hours. Results: The percentages of apoptosis at first hour were higher than those at 0 and 2 hours in 10 and 20 U/ml heparin concentrations, separately (p < 0.05). The mean percentage of apoptosis in 20 U/ml heparin levels was significantly higher than those in 0 and 10 U/ml heparin levels at 1 and 2 hours (p < 0.05) and 20 U/ml heparin concentrations at 1 and 2 hours (p < 0.05). The highest caspase-9 activity of heparin on lymphoblasts was determined at first hour in 20 U/ml heparin concentration. The mean caspase-9 activity at first hour was significantly higher than that at 0 and 2 hours in 10 and 20 U/ml heparin levels, separately (p < 0.05). The mean caspase-9 activity in 20 U/ml heparin concentration was significantly higher than those in 0 and 10 U/ml heparin concentrations at 1 and 2 hours (p < 0.05). The highest cytochrome C level at first hour was significantly higher than those at 0 and 2 hours in 10 and 20 U/ml heparin concentrations, separately (p < 0.05). The highest cytochrome C level in 20 U/ml heparin concentration at first hour. Conclusion: We claimed that heparin induces apoptosis on lymphoblasts by the activation of intrinsic pathway.

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META-ANALYSIS REGARDING SIMVASTATINUM TREATMENT IN MALIGNANT LYMPHOMA
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Background: Simvastatinum (S) is a competitive inhibitor of 3-hydrox-ide-3-methylglutaryl CoA reductase, a cholesterol and some isoprenoid products synthesis regulatory enzyme, like geranylgeranyl pyrophosphate. Despite the hypcholesterolemic effect it also has anti-neoplastic pleiotropic properties. Aims: Our purpose was to study the existing literature arguments for the use of simvastatinum as an adjuvant therapy in malignant lymphoma. Methods: We analyzed the 10 existing studies in MEDLINE, in January 2011 regarding S therapy in lymphoma. Our study included: the types of lymphoma where it was used, therapeutic action, the results of the experimental studies and its experience in clinical study usage. Results: S was administrated in various lymphoma subtypes: a mouse model of Hodgkin’s lymphoma (HL), Epstein-Barr virus (EBV)-driven lymphoma cells, Waldenstrom macroglobulinemia (WM) cells, adult T-cell leukemia cells (ATL), chronic lymphocytic leukemia (CLL) cells and in lymphoma patients. S induced caspase-related apoptosis via depletion of prenylation-substrates in several HL-cell lines. S induces apoptosis, C2/M cell cycle arrest, accumulation of p21(Waf1/Cip1), and effectively inhibits hyperproliferation of human Namalwa Burkitt lymphoma cells that display general apoptosis resistance and hyperproliferation. S inhibited cell growth, survival and IgM secretion on WM cells by inhibiting prenylation of geranylgeranylated proteins. S binds to the inserted domain of leukocyte function antigen 1 and inhibits its function, resulting in down-regulation of nuclear factor kappaB activity and induction of apoptosis of transformed B cells and EBV-transformed lymphoblastoid cell lines. S blocks the interaction of adhesion molecules that are important for cell-cell interactions including those between EBV-transformed B cells. S decreased Akt and extracellular signal-regulated kinase mitogen-activated protein kinase pathways. In ATL, statins inhibited geranylgeranylation of small GTPases Rab5B and Rac1. In CLL cells S induced apoptosis concurrently with lowering of BCL-2/BAX ratio; its pro-apoptotic effect is tumor-specific, not affecting normal lymphocytes. Combinations of simvastatin + fludarabine and simvastatin + cladribine had a synergic effect in inducing apoptosis. In S lactone inhibited P-glycoprotein mediated rhodamine 123 transport in a murine monocytic leukemia cell line that over-expresses the multi-drug resistance protein 1a/b. In vitro S induced apoptosis in a dose- and time-dependent way. In a cell line model for HL it effectively impaired tumor growth. It delayed development of EBV lymphomas and prolonged survival of animals. It induced inhibition of proliferation, had cytotoxic effect and produced apoptosis of WM cells. Furthermore, S enhanced the cytotoxicity induced by bortezomib, fludarabine and dexamethasone. Statins hinder the survival of ATL cells and induce apoptotic cell death. In combination with dexamethasone and doxorubicin, statins have a chemo-sensitizing effect and the patients with relapsed lymphoma can be treated with a dose-scaling regimen of simvastatin for 7 days followed by CHOP. High-dose simvastatin given immediately prior to chemotherapy was safe and tolerable up to a dose of 15 mg/kg/day. Summary: S inhibits geranylgeranylation - a critical process for the regulation of lymphoma tumor cell survival and proliferation. The pro-apoptotic effect of S is tumor-specific, not affecting normal lymphocytes. S has a chemo-sensitizing effect, useful in patients with relapsed lymphoma. By the P-glycoprotein inhibition it can reduce the multi-drug resistance.

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USING A NEW INTEGRATIVE APPROACH WITH SIXTEEN REFRACTORY CASES IN PEDIATRICS HEMATOLOGY/ONCOLOGY AT KING ABDULAZIZ UNIVERSITY HOSPITAL IN SAUDIA
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Background. The goal of health care is to find the most effective and cost-effective approach to prevention, health promotion, and treatment of illness, considering the use of both conventional medicine and complementary and alternative medicine (CAM) therapies. Integrative oncology, the use of CAM in conjunction with standard medical treatment, seeks to improve the supportive care available to patients, while also determining through scientific clinical trials which adjuvant CAM therapies are medically sound, effective, and compatible with standard chemotherapy and radiation. Aims. To report the effectiveness and safety of a natural experimental agent used with conventional therapy in sixteen relapsed cases with different hematological malignancy who were refractory to conventional treatment. Methods. A phase I controlled clinical trial to use the Novel Experimental PM 701 combined with conventional chemotherapy in induction and maintenance phases in the treatment of refractory and relapsed cases in Pediatric Hematology/Oncology, in a period of 2005 to 2009, treated at King Abdulaziz University Hospital, Jeddah, Kingdom of Saudi Arabia. This natural agent has been fully studied with animal models and tissue cultures at the King Faisal Medical Research Center’s laboratory at King Abdulaziz University and showed potent selective apoptotic effect on cancer cells, effective and safe in animal models. Results. All the sixteen cases showed complete remission through induction phase, by using the novel experimental PM 701 combined with conventional therapy. Fifteen cases alive, with eleven patients off treatment. One patient with known case T-ALL died after a year refractory to the treatment; due to the shortage of supply of the experimental PM701 while in maintenance. Summary. We report in this phase I clinical trial a successful, effective, and safe management with the combination of conventional therapy with experimental medicine (PM 701) in the treatment of sixteen refractory and relapsed cases in Pediatric Hematology/ Oncology. Complementary and alternative therapies need further control studies in the future to prove the efficacy and safety to be used on oncology patients.

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APOTOPSIS INDUCTION MEDIATED BY HUMAN PARVOVIRUS B19 NON-STRUCTURAL PROTEIN (NS1) IN HUMAN ERYTHROLEUKEMIA CELL LINE THROUGH ACTIVATION OF PS3 GENE

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Background. Many human leukemias do not express detectable levels of p53. It has been demonstrated that loss of p53 gene expression is likely to be an important step in leukemogenesis. This could be result from the loss of proper trans-acting factors or mutational/epigenetical silencing of cis-acting elements in regulatory regions of the gene. Apoptosis induction in leukemic cells by agents which trigger cancerous cells specifically is an active area in the field of treatment of leukemia. Aims. The objective of this in vitro study was to determine the application of NS1 protein of parvovirus B19 to induce p53 activity and apoptosis in K562 cell line as a p53-null erythroleukemia cell line. Methods. We have developed a lentiviral vector system to deliver the NS1 gene into K562 cells. Using qRT-PCR and protein analysis, we determined levels of NS1 gene induced mean of 63% of K562 cells to undergo apoptosis of K562 cell line by lentiviral vector was resulted in 84% efficiency of well as annexin-V and caspase-3 analyses were performed to assess K562 cells. Results. Using qRT-PCR and protein analysis, we determined levels have developed a lentiviral vector system to deliver the NS1 gene into K562 cells. Results. Using qRT-PCR and protein analysis, we determined levels

INFLUENCE OF NOVEL 5-AMINO-1,2-DIHYDROPYRROLE-3-ONE DERIVATIVE WITH ANTIPROLIFERATIVE ACTIVITY ON THE RAT BLOOD CELLS PARAMETERS AFTER TWO MONTH ADMINISTRATION IN THERAPEUTICAL DOSE

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Background. According to the preliminary studies the 5-amino-1,2-dihydropyrrole-3-one derivative (DD-1, 1-(4-2-ethylophiline)-4-(benzo(d)thiazol-2-yl)-5-amino-1,2-dihydropyrrole-3-one) suppresses proliferative activity of the tumor cells (HT-15 Colon Cancer, UACC-62 Melanoma, A549/ATCC Non-Small Cell Lung Cancer, SR and K-562 leukemias et al.). DD-1 was designed as inhibitor of ATP-binding sites of protein kinases and synthesized by scientific production of Chemical-Biological Centre of Taras Shevchenko National University of Kyiv. The aim of the study was determination of DD-1 effects on blood cells parameters in healthy rat and under the action of 1,2-Dimethylhydrazine induction of colon tumors after two month administration. Methods. The outbred male rats was divided into four groups (10 rats in each group): I - control group (treated 0.1 ml of the sunflower oil containing 15% of DMSO per os daily); II - group treated with 1,2-Dimethylhydrazine (20 mg/kg in saline subcutaneous every week, for induction of colon tumors, and 0.1 ml of the sunflower oil containing 15% of DMSO per os daily). III - group treated with DD-1 (2.3 mg/kg in 0.1 ml of the sunflower oil containing 15% of DMSO per os daily and 0.1 ml of saline subcutaneous every week), IV - group treated with DD-1 (2.3 mg/kg in 0.1 ml of the sunflower oil containing 15% of DMSO per os daily) and 1,2-Dimethylhydrazine (20 mg/kg subcutaneous every week) during two month. A DD-1 dose of 2.3 mg/kg corresponds to the 100% inhibition of tumor cells proliferation in vitro. Rat blood cells parameters were determined by routine method. Results. It is shown that DD-1 in the dose of 2.3 mg/kg does not effect erythroid parameters (erythrocyte counts, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC)) and leukocytes vs. control group after two month administration, 1,2-Dimethylhydrazine does not change none of blood cells parameters after two month administration. In IV-group treated with DD-1 and 1,2-Dimethylhydrazine MCH was decreased (10.65±0.01, P<0.05) and MCHC has a tendency to decrease (320.29±151, P=0.07) vs. control group (19.43±0.16, 335.69±3.66, respectively) after two month administration. The other parameters did not change. Conclusions. DD-1 does not affect the blood cells parameters in healthy rat in indicated dose and does not restrict application of this compound as a potential anti-tumour drug.
oxophoretic capacity of the blood. Classically, the reduction in RBC mass is obtained with bloodletting by phlebotomy, in order also to induce hyposiderosis and iron-restricted erythropoiesis. Aims. In this study we report the preliminary data obtained with a new procedure of selective erythropoiesis, called SEMEA: Selective Micro Erythrocytes Apheresis, that we have designed in order to remove from the circulation mainly the microcytes. We have evaluated if this innovative and original method could own the characteristics of the traditional phlebotomy, to take away an increased red cell mass, and if it is also able, respect to non-selective erythropoiesis, to take a selected population of cells: the microcytes. Methods. During 2010, 10 male patients affected with PV, aged 64.5±7.94, were treated, after informed consent, by SEMEA, each underwent to a cycle of 6 apheresis in 30 days. Therapeutic procedures were performed with cellular separator Haemonetics MCS+, using the 971E kit for collection of PBSC, but opportunely modifying some parameters: a) AC ratio from 1:9 to 1:12; b) start collection set to 70%; c) detected RBCs set at 12%; d) removed volume of RBCs concentrates per cycle: 40 mL; e) ratio from 1:9 to 1:12; f) removed volume of RBCs concentrates per cycle: 40 mL; g) no recycling; h) changing the original bowl with another of 225 mL. Results. Observed data are expressed as mean±SD. Before our treatment: Hct = 48.98±4.4%, Hb = 14.3±1.51 g/dL, RBC = 6.83±0.2106/μL, MCV = 71.25±7.94 fl, RDW = 17.3±1.51%. Observed values on all collected units: RBC = 3.12±0.26, MCV = 69.75±6.65, RDW = 17.35±1.24. After SEMEA: Hct = 46.78±3.94, Hb = 13.9±1.71, RBC = 6.05±0.1, MCV = 72.5±6.05, RDW = 16.85±1.94. Conclusions. At the end of this first experimental course of treatment, we observed that: 1) the procedure was effective in reducing polycythaemia; 2) slight increase of MCV in vivo; 3) the MCV of collected RBCs was always 2-3 fl lower than basal MCV of the patient; 4) not significant change in Hb before and after SEMEA. Moreover the treatment has been well tolerated, in fact patients showed no adverse reactions despite the longer duration of the procedure compared to standard therapeutic erythropoiesis (80 vs 15 minutes). The original method, in addition to the reduction in RBCs count, has the advantage of affecting the real pathological cellular fraction of PV, as demonstrated by decreasing of RDW. On the basis of our results, we are encouraged to propose SEMEA to patients affected with PV showing a market polyglobulia with hyposideremic anisopoikilocytosis after a series of bloodletting by phlebotomy.

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**DATA OF ADVIA 120® RBC MATRIX ARE USEFUL TO DISCRIMINATE B12/FOLATE DEFICIENCY, CHEMOINDUCED ANDBeta THALASSEMA MINOR ANAEMIA**

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Background. In automated peripheral blood cell analysis, ADVIA® technology (Siemens) provides a lot of information regarding also red blood cells (RBC). Among these information, those regarding red blood cells (RBC) contained in RBC matrix might be useful to define different aetiology of anaemia. Aims. Aim of this study is to define if information contained in RBC matrix is useful to define different aetiology of anaemia, before that data regarding iron balance, B12, folates, DAT and IAT and haemoglobin electrophoresis are available. Methods. We evaluated 148 patients with moderate/severe anaemia. M/F was 57/91. Median age was 65 years (R23-87). 14 patients presented B12 and folates deficiency anaemia (9%), 68 with chemoinduced anaemia (45%), 9 with beta thalassemia minor (6%), 57 with sideropenia (38%). Data were analyzed using RBC matrix of ADVIA 120® automated blood cell analyzer. In this matrix volume of RBC is plotted with haemoglobin concentration of RBC. In this matrix there are 9 box (RBC macrocytic/hypochromic, macro/normo, macro/hyper, normo/hypo, normo/normo, normo/hyper, micro/hypo, micro/normo, micro/hyper). In each box ADVIA 120® provide absolute and percentual number of RBC with above mentioned characteristics. Results. Among 14 patients with B12 and/or folate deficiency, 11 (78.6%) presented RBC simultaneously macrocytic/hypochromic>2% and normocytic/hyperchromic>2%. In remaining 3 patients with anaemia without B12 and/or folate deficiency, a Chi Square Yates corrected of 46.962 (p<0.0001), with an Exact Fisher test with p<0.0001, with a sensitivity of 78%, a specificity of 92% and a predictive negative value of 97.6%. Among 68 patients with chemotherapie anaemia, 10 (14.7%) presented RBC macrocytic/hyperchromic>2%. In remaining 80 patients only 3 showed the same characteristics, with a Chi Square Yates corrected of 4.2 (p<0.04), with an Exact Fisher test with p<0.038, with a sensitivity of 15%, a specificity of 96% and a predictive positive value of 77%. Among 9 patients with beta thalassemia minor, 8 (89%) presented RBC simultaneously microcytic/hypochromic>2% and microcytic/normochromic>2%. In remaining 159 patients no patient showed the same characteristics, with a Chi Square Yates corrected of 113.8 (p<0.0001), with an Exact Fisher test with p<0.0001, with a sensitivity of 89%, a specificity of 100% and a predictive positive value of 100% and a predictive negative value of 99%. Summary and conclusion. ADVIA 120® RBC matrix is useful to define B12 and/or folate deficiency anaemia (RBC simultaneously macrocytic/normochromic>2% and normocytic/hyperchromic>2%), chemorelated anaemia (RBC normocytic/hyperchromic>2%), beta thalassemia minor (RBC simultaneously microcytic/hypochromic>2% and microcytic/normochromic>2%). These data need confirmation on a larger cohort of patients.

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**JUVENILE HAEOMOCRATOSIS**

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Juvenile haemochromatosis (JH) is the most severe form of inherited iron overload usually affecting either sex before the age of 30. Sadly the first manifestation of JH can be sudden cardiac failure. In Europeans the disease is associated with heart disease, diabetes, infertility and liver disease. In Asian patients JH associated heart disease is rare. The genetic picture with JH is not simple. JH is usually attributed to mutations in either hemojuvelin (HJV) or hepcidin (HAMP). However JH can also be due to combinations of mutations in HFE and hepcidin or transferrin receptor 2 (TFR2) and HFE. JH rarely results from mutations in TFR2 alone. Hence in the majority of cases autosomal recessive inheritance of mutations in HJV, HAMP or TFR2 explain JH. Rarely combinations of mutations (digenic inheritance) are the explanation. Most commonly JH is due to mutations in the HJV gene. Mutations in HAMP gene are rarer and tend to be more severe. Nevertheless both conditions are rare. In Europeans the most common HJV mutation is G320V and in Asians G99R. The range of mutations is such that detailed genetic analysis is required for individual families. The age of onset may be determined by the ethnic background and the location of the mutation in HJV. Mutations in highly conserved residues result in severe disease. As part of the haemochromatosis screening service that has been set up in Oxford we have received requests to investigate the genetic basis of iron loading in a number of C282Y negative patients and their families. We have had 80 requests from referring departments to perform mutation screening in the genes encoding ferroportin, hemojuvelin, hepcidin and transferrin receptor 2. Apart from one case all had unexplained increased serum ferritin, some elevated transferrin saturation. Here we describe those requests relating to JH. In the first case the proband sadly died and it was a consequence of the postmortem that the diagnosis of JH was made. Autosomal recessive inheritance of mutations in HJV and HAMP was found and the family were initially denied further screening. The proband was found to be homozygous for the common G320V HJV mutation. The second case was a family of Asian origin living in the UK where the proband was found to be homozygous for the common Asian mutation G99R. The family was not related to families living in the Midlands with the same mutation which had been described previously. The third case is a British male with congestive heart failure. He was referred to Oxford for further screening. He was found to be homozygous for the rare C80R HJV mutation that had previously only been described in a compound heterozygote living in France. From our findings we strongly recommend that any patient with unexplained raised serum iron parameters, found to be negative for HFE mutations is screened for mutations in HJV, HAMP, TFR2 and ferroportin.

**1554**

**FERROPORTIN DISEASE, 4 NOVEL MUTATIONS**

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The different genetic forms of HH share as their pathogenic mechanisms the deficiency or dysregulation of the hormone hepcidin or defects involving the hepcidin receptor, ferroportin. Ferroportin is the sole iron exporter in enterocytes, macrophages and hepatocytes. Mutations in the ferroportin gene can cause a significant decrease in circulating iron release into plasma. Mutations in the ferroportin SLC40A1 gene cause iron over-
load syndromes with AD inheritance. Ferroportin mutations result in spectrum of phenotypes, depending on the functional alteration of the protein. At one end of the spectrum, loss of function mutations, leads to increased macrophage iron and elevated serum ferritin, but normal transferrin saturation, and is known as “ferroportin disease”. At the opposite end of the spectrum a distinct phenotype, similar to classical haemochromatosis, is associated with gain of function mutations. These mutations cause ferroportin resistance to the effects of hepcidin by preventing internalisation of the hepcidin-ferroportin complex. From our cohort of patients that had been sent in for ferroportin gene screening 2 known and 4 novel mutations were identified. The novel mutations in exon 5, W158C, D135A and D157Y are all in highly conserved amino acid residues. Four different mutations have been found at codon 157. The mutation D157Y was found in an individual from north India. A molecular mechanism for mutations in this position can be explained by the D157G mutation in ferroportin leading to hepcidin-independent binding of JAK2 and ferroportin downregulation. Two previous ferroportin mutations had been found in India and Thailand and these were V162del and C326Y. They are rare mutations in this ethnic group. The mutation found in exon 8, H507R is a highly conserved amino acid residue which is predicted to be in the transmembrane domain (codons 492-514 in exon 8).

A published mutation p.Y501C has been found in the same transmembrane domain, this causes non-classical ferroportin disease. All individuals in the family were also heterozygous for C282Y mutation. Ferroportin disease is a clinically and genetically heterogeneous iron overload syndrome. Our results demonstrate the importance of screening patients found to lack HFE mutations for ferroportin mutations.

### 1555

**TRANSFERRIN POLYMORPHISM AND ANEMIA IN HIV**

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**Background.** Anemia is a common finding in developing countries, especially among women. Moreover, anemia is frequently observed in HIV, with prevalence up to 80% in patients with asymptomatic infection and in as many as 90% in those with AIDS. Importantly, anemia is associated with decreased quality of life and shortened survival. The main cause of deaths in HIV is opportunistic infection. Therefore we explored if anemia, and more especially if iron related parameters correlated with the risk to get opportunistic infections. More specifically we examined the impact of the genetic polymorphism of human transferrin (TF) since; it has been reported that the transferrin CD phenotype was associated with variations in certain measures of iron status. In Caucasians the common (CC) TF phenotype is almost found exclusively. Aims: To determine the prevalence and risk factors of anemia and the influence of HAART on anemia. To explore the relationship between transferrin polymorphism, anemia and the frequency of opportunistic infections. Methods: We conducted a cross-sectional study among 200 HIV positive and 50 HIV negative women in Butare University Teaching Hospital in Rwanda. Informed consent was obtained from all women. Complete physical examination was carried out and WHO HIV disease stage classification, hematological parameters, CD4 count and ferritin were determined. TF phenotypes were determined using starch gel electrophoresis. Results: The prevalence of anemia was significantly higher among HIV positive women (29%) than in HIV negative women (8%), p<0.001. Risk factors for anemia (OR and 95% CI shown in brackets) were lower body mass index (OR:3.8[2.4-4.1]), Zidovudine use (OR:1.14[1.01-1.29]), lack of HAART (OR: 1.44 [1.21-1.67]), oral candidiasis (OR:1.4[1.2-1.6]), pulmonary TB (OR:1.8[1.7-2.2]), cryptococcal meningitis (OR:1.6[1.21-1.8]), Pneumocystis jiroveci pneumonia (OR:1.41[1.20-1.65]) and CD4 lymphocyte count <200 cells/μL (OR:2.4[1.2-1.6]). The mean hemoglobin of 10.9 ± 1.6 g/dL at HAART initiation significantly increased to 13.5±1.5g/dL (p<0.001) for the CD4 count <350 cells/μL. The homzygous TF DD phenotype was not found. Subjects with TF CD phenotype had a significantly lower frequency of opportunistic infections than subjects with TF CC phenotype, 24% and 46% respectively (p=0.026). Subjects with TF CC phenotype had significantly lower values for serum beta 2 microglobulin (P=0.004) and transferrin (P=0.006) compared with TF CC subjects. There was a trend towards lower serum iron concentrations in subjects with TF CD (P=0.08). Overall hematological parameters, ferritin, transferrin saturation, CRP and CD4 count did not differ according to TF phenotype. Conclusions: The prevalence of anemia is important and its prevalence varies among different groups depending on factors such as ethnicity, lifestyle or fitness, the appropriateness of current WHO definition of anaemia in the elderly may therefore be questioned. We have evaluated peripheral blood parameters from 1,244 individuals (908 women aged 18 to 101 years and 816 men aged 40 to 96 years), who were seen at the University of Heidelberg Medical Center in the absence of a known haematological history. Patients with a known malignant haematological or oncological disease, with chronic infec-tion or inflammation were excluded. Patients with disorders affecting the kidneys, thyroid or stomach as well as patients with a bleeding history, hemolysis or who had been previously diagnosed to have anemia were excluded from this study. Anemia was defined as hemoglobin levels for men beyond the age of 70 and for women beyond the age of 80 were found to fulfil the WHO criteria for the diagnosis of anaemia. While in our cohort already ~ 20% of men and women between 60-69 years of age were by definition anemic, these numbers were steadily increasing to up to 63% in females and to 76% in males beyond the age of 70. Based on the results of our study and in accordance with the literature to this topic we suggest age-adjusted criteria for the diagnosis of anaemia in the elderly in conjunction with a geriatric assessment.

### 1556

**AGE-RELATED CHANGES OF PERIPHERAL BLOOD COUNTS IN MAN**

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Anemia is becoming a common concern in geriatric health. Even though its prevalence varies quite significantly among different groups depending on factors such as ethnicity, lifestyle or fitness, the appropriateness of current WHO definition of anaemia in the elderly may therefore be questioned. We have evaluated peripheral blood parameters from 1,244 individuals (908 women aged 18 to 101 years and 816 men aged 40 to 96 years), who were seen at the University of Heidelberg Medical Center in the absence of a known haematological history. Patients with a known malignant haematological or oncological disease, with chronic infection or inflammation were excluded. Patients with disorders affecting the kidneys, thyroid or stomach as well as patients with a bleeding history, hemolysis or who had been previously diagnosed to have anemia were excluded from this study. Anemia was defined as hemoglobin levels for men beyond the age of 70 and for women beyond the age of 80 were found to fulil the WHO criteria for the diagnosis of anaemia. While in our cohort already ~ 20% of men and women between 60-69 years of age were by definition anemic, these numbers were steadily in-creasing to up to 63% in females and to 76% in males beyond the age of 90. Based on the results of our study and in accordance with the literature to this topic we suggest age-adjusted criteria for the diagnosis of anaemia in the elderly in conjunction with a geriatric assessment.

### 1557

**HEPCIDIN, A NEW HORMONE OF IRON HEMOSTASIS**

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**Background.** Anemia of chronic disease (ACD) results from 3 major processes: slightly shortened red cell survival, impaired reticuloendothelial system iron mobilization, and impaired erythropoiesis. Hepcidin is an acute-phase protein with specific iron regulatory properties, which, along with the anemia seen with increased hepcidin expression, have led many to consider it the major mediator of ACD. Hepcidin is a principal iron regulatory hormone and its expression is stimulated by cytokines. The aim of this study was to determine serum levels pro-hepcidin in ACD anemia. Methods: The study included 115 patients, 72 males and
46 females. Anemia was defined as hemoglobin below 12 g/dl in females and 13 g/dl in males. We have 68(37.6%) anemic patients, 57(31.4%) have ACD, 17(14.4%) IDA, 11(9.3%) ACD–IDA and 50(42.4%) no anemic patients. TNFα, interleukin IL6 and levels were determined by Immulite 1000. DRG ELISA kits were used for prohepcidine determinations. Independent Sample Test, Anova test, Chi-Square Tests was used for statistical analysis. Results: Serum prohepcidin, IL6, TNFα concentration (r = 0.226, p = 0.041 r = 0.309 p = 0.006 respectively) and IL6 have a strong correlation with serum ferritin and TNFα (r = 0.07 p = 0.50) and are not related to HbC. We suggest that hepcidin is a principal iron regulatory hormone in ACD and its expression is stimulated by IL6 cytokine. Serum prohepcidin concentration is the best marker to diagnose ACD.

HEMOGLOBIN EXTREMADURA [?64(E8)GLY>SER + ?133(H11)VAL>LEU]: A NEW MOLECULAR ANALYSIS AND CORRECTION OF A HAEMOGLOBIN PREVIOUSLY PUBLISHED


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Unstable hemoglobin (Hb) variants account for 9.5% of structural hemoglobinopathies. The majority of these unstable variants are the result of gene point mutations resulting in the substitution of a single amino acid by another. The presence of two mutations in the same allele is infrequent: of the 781 variants of the beta globin cluster described, only 32 are due to two point mutations (4.1%). Hemoglobin (Hb) Extremadura is a structural variant that is included within the so-called unstable Hb. It was described in 1989 by Villegas et al., employing the most pioneer- ing techniques available at that time, reverse phase HPLC to separate the abnormal chain (βX) digesting it with trypsin and analysis of the fragments with an automatic analyzer. The carrier was a 27-year-old who had a slight slight splenomegaly and hemolytic anemia (Hb 11.0 g/dl, PCV 31.5%, RBC 5.4×10⁶/L, MCV 92fl, MCH 32.1 pg, MCHC 34.9 g/dl, Retis 5%), Heinz bodies (+). The Extremadura is electrophoretically silent Hb on cellulose acetate at alkaline pH, isoelectric focusing (IEF) and ion exchange HPLC, except on citrate agar at acid pH observed a diff- use band between Hb S and Hb C. By reverse phase HPLC eluting behind the chain βX (BA, βX and αβ), αα). Over these 20 years have main- tained the value of αβ (Hb 9.0 g/dl, PCV 34.2%, RBC 5.3×10⁶/L, MCV 106.4 fl, MCH 32.5 pg, MCHC 30.6 g/dl, Retis 2.5%). She has had a daughter, who also presents mild splenomegaly and signs of minimal hemolysis (Hb 12.1 g/dl, PCV 38.0%, RBC 3.5×10⁶/L, MCH 109.7 fl, MCHC 34.9 pg, MCHC 31.8 g/dl, Retis 2.2%), of Heinz bodies. At that time it made the study of globin chains by reverse phase HPLC, showing abnormal β chain compatible with the mother. It was decided to complete the study by sequencing the gene β globin, by reverse phase HPLC eluting behind the chain βX (BA, βX and αβ), αα). The 20 years have maintained the value of αβ of the mother and daughter, being a double mutation in the CD 64 of the 2nd exon GGC→AGC (Gly→Ser) and the CD 153 of the 3rd exon GTG→CTG (Leu→Val). The correct molecular characterization of these two point mutation Hb variants facilitates the understanding of how they influence changes in Hb stability, solubility and function (oxy- gen affinity), ultimately responsible for the clinical manifestations of the hemoglobinopathies, and permits the prediction of possible interactions with other Hb mutations, thus allowing more accurate genetic counsel- ling techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar. Sicking test and tests for HHb inclusion bodies were also performed. Haemoglobin A2 was also quantified by column micro chroma- tography and serum ferritin levels were measured by micro ELISA tech- nique. Only 6 individuals were found to be heterozygotes for Hb Setif and two cases were compound heterozygotes of Setif / beta thalas- saemia. In heterozygotes the percentage of Hemoglobin Setif was from 16.2-17.4% and Hb A2 was within the normal limits. The combination with beta thalassemia was characterized by low hemoglobin Setif lev- els that varied as low as 2.2 to 2.7% unlike of the double heterozygos- ity. Both heterozygotes and compound heterozygotes did not manifest any clinical problem. Our data indicate that Hemoglobin Setif is rare in our country and clinically silent. Identification is important for genetic counseling of families since the coinheritance with beta thalassemia does not affect the hematological and clinical manifestations of the carriers. The fact that levels of hemoglobin Setif in compound heterozygotes with beta thalassemia genes are seen in low as seen in two of our cases is an inter- esting finding.

HEMOGLOBIN SETIF IN NORTHERN GREECE. EPIDEMIOLOGICAL SURVEY

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Hemoglobin Setif (α 94 Asp-Tyr) was first described in 1972 in Algeria and since then several cases have been reported in families in Iran, Lebanon and other Middle Eastern states. The substitution in Hb Setif is in the α1β2 contact region of the molecule. This site of amino acid substitution in Hb Setif is an important contact point between α and β chains in the oxyconformation. Oxygen affinity is only slightly reduced nevertheless mild instability is produced. It has been reported that the most remarkable property of Hb Setif is its capacity to induce pseudo-sickling of red cells in vitro. This does not have any clinical significance. Identification of Hb Setif is made by hemoglobin electrophoresis and iron exchange high pressure liquid chromatography (HPLC) as well as DNA studies. Hb Setif moves to the position of Hb S on starch gel and cellu- lose acetate at both alkaline and acidic pH. Hb Setif and Hb A separate due to their different carbohydrate content on HPLC. The percentage of Hb Setif is 12-15% in heterozygotes, and it is reported that it could be as high as 30% in a compound heterozygotes with alpha and beta thalassemia muta- tions in the same person without any clinical consequences. The aim of this study was to investigate the incidence of Hb Setif in Northern Greece. A total of 78.254 subjects were screened for hemoglobinopathies in our prevention unit that covers the regions of Central and Western Macedonia in Northern Greece with a population of around 2.5 million. The carrier identification was carried out by a standard scheme, which included, CBC and red cell indices using the Coulter ONYX, a Cation Exchange HPLC variant system (Bio-Rad, Variant β-Thalassemia Short Program), to determine HbA, HbA2 and HbF levels. The different abnor- mal structural hemoglobins were investigated by electrophoretic tech- niques both at alkaline pH on cellulose acetate and at acid pH on citrate agar. Sicking test and tests for HHb inclusion bodies were also performed. Haemoglobin A2 was also quantified by column micro chroma- tography and serum ferritin levels were measured by micro Elisa tech- nique. Only 6 individuals were found to be heterozygotes for Hb Setif and two cases were compound heterozygotes of Setif / beta thalas- saemia. In heterozygotes the percentage of Hemoglobin Setif was from 16.2-17.4% and Hb A2 was within the normal limits. The combination with beta thalassemia was characterized by low hemoglobin Setif lev- els that varied as low as 2.2 to 2.7% unlike of the double heterozygos- ity. Both heterozygotes and compound heterozygotes did not manifest any clinical problem. Our data indicate that Hemoglobin Setif is rare in our country and clinically silent. Identification is important for genetic counseling of families since the coinheritance with beta thalassemia does not affect the hematological and clinical manifestations of the carriers. The fact that levels of hemoglobin Setif in compound heterozygotes with beta thalassemia genes are seen in low as seen in two of our cases is an inter- esting finding.
Conclusions. Automatic sequencing is a great important method for the clinical diagnosis of non-deletional α-thalassemia syndromes. Homozygous cases show a severe hypochromic anemia, with a lower Hb and a decrease of VCM, which is supported by the literature on the subject. The VCM abnormally high for the case of homozygous Hb Agrinio could be explained by the fact that the individual has received a transfusion recently. In patients II.3 of family C and II.4 of family B, the VCM is low considering that present Agrinio heterozygous Hb. However, this could be explained by iron deficiency anemia associated. Hb Agrinio heterozygous causes a thalassemia trait while in homozygotes behave as an intermedia thalassemia because they are affected both α genes, which have a higher rate of gene transcription compared to α1. This Hb is hyperunstable, why suffer instantaneous postradutional precipitation. Here, a residue highly conserved in evolution, essential to create the hydrophobic environment necessary for distal heme binding is affected, disrupting contacts with residues 55, 58, 59 and 101 of the α chain. The diagnosis of less common variants of this disease is essential to be applied to genetic counselling and prenatal diagnosis, reducing health and social costs associated with processing of these pathologies.

1651

FIRST CASE OF HEREDITARY ATRANSFERRINEMIA IN SPAIN

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Background. Hereditary atransferrinemia (OMIM 209300, Orphan number ORPHA1195) was first described in 1961 as a rare autosomal recessive metabolic disorder characterized by microcytic hypochromic anemia due to a functional deficiency of transferrin. Transferrin is the main plasma iron-binding protein encoded by the gene TF. In atransferrinemia, there is a reduction in delivery of iron to erythroid cells in the bone marrow, reduced haemoglobin synthesis, increased iron absorption, and severe iron overload of parenchymal organs. The disease has been reported in only 12 patients from 10 families worldwide. Just four of these families have been studied at the molecular level. Aims. Here, we report a new patient with this rare disorder, the first known case in Spain and the 15th patient to be reported in literature. We aim to characterise this family at the genetic and molecular level. Methods and Patients. The pre-treated transferrin levels of the proband were extremely low (12.5 mg/dL normal values: 202 - 336 mg/dL). The parents and other relatives had low transferrin levels (value range 124 - 184 mg/dL) as predicted for healthy carriers. Blood samples were taken from the patient and relatives and DNA was extracted. Lymphocytes were isolated using the Ficoll method and grown in cell culture. Cytokine treatment was added to induce transferrin expression and RNA was isolated. Primers were designed for genomic amplifications (PCR) and transcriptional analysis (RT-PCR and qPCR). PCR fragments were sequenced by the conventional Sanger method and mutational analysis was performed using Mutation Surveyor DNA Variant Analysis Software (SoftGenetics). Results. Genetic analysis of the transferrin gene in our case revealed common polymorphisms, silent mutations and three novel variations in intronic regions. In addition, the proband and her mother had a new missense mutation in heterozygous state; NM_0010653: c. [1561C→A]→[A]. NP_0011084.1:p.[Ala418Glu]→[Glu]. This mutation is located in a conserved region of the protein. Paternal genetic analysis did not reveal any mutation in the coding region. The patient is currently being treated satisfactorily with periodic infusions of purified apotransferrin. Summary/Conclusions. The detected Ala418Glu amino acid substitution predicts a structural change in the protein that probably affects transferrin function. As Atransferrinemia is an autosomal recessive disorder, we propose a second mutated allele inherited from the father (healthy carrier). We are currently studying the TF promoter region and the implication of intrinsic variations in RNA missplicing.

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1652

EVIDENCE OF PROGNOSTIC VALUE OF RETICULOCYTE COUNT AND RETICULOCYTE HEMOGLOBIN CONTENT IN SICKLE CELL DISEASE

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Background. Reticulocytes are generally accepted to be young red blood cells. Whenever red cell formation in the bone marrow is active, the proportion of reticulocytes in blood increases. To date, reticulocyte hemoglobin content (Ret-he) is the most widely studied of the reticuloctyes indices. The hemoglobin content is considered to be constant throughout the lifetime of erythrocytes and circulating reticulocytes undergo several changes during the life span of the red cell, which is reflected in the reticulocyte parameters. The decrease of reticulocyte parameters is believed to be a sign for improved erythropoiesis hypothesis. The decrease of the Reticulocyte count and the increase of Ret-He could be used as indicative indices for the severity of sickle cell disease.

1653

MTHFR GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH HEMATOLOGIC ALTERATIONS IN WOMEN WITH RECURRENT PREGNANCY LOSSES

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Results. Recurrent pregnancy loss (RPL) is defined as three or more consecutive spontaneous abortions prior to the 20th week from the last menstrual period. It is a multifactorial condition. Low concentration of serum folate was observed in carriers of MTHFR 677TT genotype and it could impair the hemoglobin concentration. The effects of haplotypes for MTHFR 677C>T and 1298A>C polymorphisms in the hemoglobin parameters are unknown. Aims. To investigate the relationship between MTHFR 677C>T and 1298A>C polymorphisms and hematologic alterations in women with recurrent pregnancy losses (RPL). Methods: Two
hundred and forty two non-pregnant women with three consecutive losses prior to 20 weeks of pregnancy and 257 healthy fertile non-pregnant women (group 3) who had at least two children and no known pregnancy losses were studied. The primary RPL group was made up of women who had three or more consecutive losses without carrying a fetus to viability (group 1, N=109) and in the secondary RPL group, women were included who had three or more consecutive losses and with at least one viable pregnancy (group 2, N=183). The hemogram was obtained by using a hematologic cell counter Pentra 120 ABX. Cobalamin was determined by using an Immulite (DPC Medlab) kit. Serum folate was determined by a microbiological assay. The MTHFR (677C>T and 1298A>C) polymorphisms were detected respectively by RFLP PCR and Real Time PCR. Results: Variante allele frequencies for MTHFR 677C>T and 1298A>C polymorphisms were similar in three groups (P>0.05). The frequencies of haplotypes for MTHFR 677C>T and 1298A>C polymorphisms in three groups were also similar (P>0.05). The number of blood cells (erythrocytes, leucocytes and platelets) and hemoglobin concentrations were similar in three groups according to genotypes or haplotypes for MTHFR 677C>T and 1298A>C polymorphisms (P>0.05). No difference was observed in Cbl concentrations according to three groups of genotype women for two polymorphisms (P>0.05). Women from RPL groups took supplementation with folic acid when the blood was collected, thus serum folate concentrations were similar in group 1 and 2 according to genotypes for MTHFR 677C>T polymorphism and higher than serum levels found in the control group, however in the control group, carriers of 677TT genotype with lower serum folate when compared with CT and CC genotypes. No difference was found in the serum folate concentrations in three groups according to MTHFR 1298A>C polymorphisms (P>0.05). Conclusions: The MTHFR (677C>T and 1298A>C) polymorphisms are not associated with hematologic alterations in women with RPL.

**Figure 1. Alpha globin genes analysis by Genescan.**

**1564**

**STRATEGY FOR MOLECULAR CHARACTERIZATION OF ALPHA THALASSAEMIA IN OMANI POPULATION**

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**Background.** Alpha-thalassaemia is known to be prevalent in the Sultanate of Oman. However, there are no studies that have reported molecular characterization of alpha thalassaemia from the Sultanate of Oman. Presence of Hb Barts in a neonate is pathognomonic of an underlying alpha thalassaemia. Materials and Methods. In a prospective study, cord blood samples from 7387 neonates, which showed abnormally high Hb Barts by a qualitative and quantitative high performance chromatography (HPLC) (Biorad Laboratories, Hercules, CA, USA) were subjected to Genescan studies. [Figure] Samples with a single peak, suggestive of a deletion lesion were subjected to GAP PCR for alpha-thalassaemia. Samples that showed two peaks, indicative of a non-deletional lesion were initially screened for common defects like alpha T-Saudi by Fast PCR. If negative these samples were then studied for rarer non-deletional defects by direct sequencing using ABI 3100 Genetic Analyzer®, (Applied Biosystems, Foster City, CA, USA). Results: Overall there were 4042 samples (51.58%) with normal HPLC (HbA + HbF). The remaining 3795 cases (48.42%) were associated the presence of Hb Barts, indicative of deletional type [two peaks] but 61.23% subjects showed deletional (αα-) type of alpha-thalassaemia. [Figure]. This was confirmed by GAP PCR for deletional cases whereas in non-deletional cases selective amplification and direct sequencing of both αα2 and αα1 genes was necessary. Conclusions: **The incidence alpha-thalassaemia in this cohort of neonates was 48.42%.** CBC and HPLC on a newborn sample can lead to the suspicion of an underlying alpha thalassaemia. A strategy to screen these samples with Genescan studies can help to classify the underlying defect as either deletional or non-deletional type, so that further appropriate molecular characterization can be facilitated. Capillary electrophoresis of alpha-globin genes analyzed by the Genescan software is thus the ideal intermediary step to choose an appropriate molecular method to characterize the alpha-globin genotype.

**1565**

**DEFERASIROX FOR THE TREATMENT OF TRANSFUSIONAL IRON OVERLOAD IN SICKLE CELL ANEMIA: A 2-YR PROSPECTIVE STUDY**

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Background: Majority of patients with sickle cell disease (SCD) receive repeated blood transfusions by adulthood. Because the body has no physiological mechanism to actively excrete excess iron, chelation therapy is important for the management of iron overload (IOL) and its complications, including iron deposition into the liver, heart and endocrine organs, eventual death. Deferasirox is a mild, oral iron chelator that is approved as first-line treatment of chronic transfusional IOL including SCD. Aims and Methods: Objectives of this prospective trial were to evaluate the IOL status, before and after two year-treatment with DFX, using liver iron concentration [LIC, mg/dl] by magnetic resonance imaging (MRI), MBI cardiars (Cardiac T2*, ms), serum ferritin (SF, µg/L), and to evaluate the safety and tolerability of DFX. Results: A total of 31 patients with SCD and IOL received starting dose of 20mg/kg/day of DFX. Two patients discontinued treatment at 8 and 9 months, due to pregnancy and moving to other city, respectively. One patient died at 18 months due to pulmonary infection and hemorrhagic stroke. DFX was interrupted in 3 patients due to confirmed SF levels <500 µg/L at 18-month period of treatment and DFX was not reinstated in none of them during the final 6 months of study. Twenty-five patients completed 2-year treatment. Mean ± SD age 26.9 ± 12.5y; 84% female, 90% afrodescendent, 61.3% on regular blood transfusion; medi-an (range) DFX dose over 24 months and DFX exposure were 20 mg/kg/day (15-25) and 90.5 weeks (35.6-98.0), respectively. Mean SF level (µg/L) did not significantly reduced at 12 months (p=0.052) but significantly dropped at 24 months compared to baseline [from 2344.6 to 1986.3 (p=0.040)]. Mean ± SD LIC significantly dropped at 12 months and at 24 months compared to baseline [from 13.0 ± 5.4 to 10.4 ± 6.3 (p=0.001)] and to 9.3 ± 5.7 (p<0.001), respectively. The proportion of patients with LIC levels (mg/dl) ≤7.0, >7.0-≤14.0 and >14.0 from baseline to 24 months by percentage of patients changed from 13.6% to 44.0%, 40.9% to 44.0% and 45.5% to 12.0%, respectively. In all patients, Cardiac T2* was normal (>20 ms) at baseline, 12 and 24 months of treatment. There was no significant difference between left ventricular ejection fraction values at baseline and after 12 months but this parameter significantly increased at 24 months of treatment compared to baseline [from 62.2 ± 6.0 to 64.6 ± 6.2 (p=0.02)]. The most common drug-related AEs were mild, transient diarrhea (7 pts), headache (7), nausea (5), vomiting (5), skin rash (2), increases in ALT (2), serum creatinine increases that exceeded the ULN (2). No patient experienced progressive increases in serum creatinine or renal failure. Conclusions: Our data confirm that deferasirox is effective in reducing body iron burden in transfused patients with SCD, well tolerated in pediatric and adult patients and with a clinically manageable safety profile. The availability of deferasirox as a once-daily, oral iron chelator would potentially facilitate improved compliance, and thereby reduce morbidity and mortality from iron overload.
COMPARISON OF CAPILLARY ELECTROPHORESIS TO HIGH-PRESSURE LIQUID CHROMATOGRAPHY IN THE EVALUATION OF HAEMOGLOBINOPATHIES

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The precise detection of structural haemoglobins as well as the accurate precision of Hb A2 and Hb F are very important issues in clinical laboratories. The detection and diagnosis of several haemoglobinopathies is important for the genetic counseling of couples at risk. In this prospective study we compared the high-pressure liquid chromatography method (HPLC), (Variant, Biorad) that we routinely use for diagnosis, with the automated capillary zone electrophoresis (CE), (Sebia Minicap). 126 samples were examined by HPLC and CE. 62 samples were found to be normal, 25 were heterozygous for β-Thalassaemia, 26 samples of people with sickle cell trait or patients with sickle cell syndromes, 2 were carriers of οβ-Thalassaemia, and 11 were found to be carriers of abnormal haemoglobins as E, Lepore, H, E-Sakatoon, D, Hb Questembert. For the normal samples the mean value of Hb A2 with the HPLC method was statistically significantly higher in comparison to CE method, (2.92 ± 0.44, and 2.7 ± 0.46 respectively) (p=0.00). For the samples from β-Thalassaemia carriers, the mean value of Hb A2 with the HPLC method was statistically significantly lower in comparison to CE method, (5.2 ± 0.69, and 5.47 ± 0.74 respectively) (p=0.014). For the samples from people with sickle cell trait or patients with sickle cell syndromes, the mean value of Hb A2 with the HPLC method was statistically significantly higher in comparison to CE method, (4.4 ± 0.97, and 3.63 ± 0.88, respectively) (p=0.00). Interestingly, CE method can measure the percentage of Hb H, fact that fails with HPLC method. Hb E co elutes with Hb A2 in HPLC method and Hb E-Sakatoon co elutes with Hb S. With CE method, Hb E is “identified” as Hb C and Hb E-Sakatoon as Hb G-Arab. As for Hb Lepore it is known that co elutes with Hb A2 in HPLC but in CE it is separated. The carrier of Hb Questembert was not identified neither in HPLC nor in CE. Both methods provide automated detection of variant haemoglobins. The use of HPLC has the advantage of a broad literature with the use also of classical electrophoresis at alkaline and acid gels. The use of CE in combination with the HPLC could become a useful tool in clinical laboratories.

NEW PARAMETERS FOR THE DETECTION OF MEGALOBLASTIC ANAEMIA

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The classical flow charts used widely for the diagnostic approach of anaemia due to Vitamin B12 (B12) deficiency or anaemia due folate deficiency includes the Mean cell Volume of the red blood cells (MCV) as one of the key tests for the suspicion of these diseases and the differential diagnosis of anaemia. Only around half of the patients with B12 deficiency (B12) or folate deficiency have high MCV, in many of the situations because the coexistence of other causes of anaemia. The Coulter LH 780 hematology analyzer (Beckman Coulter) has the ability to measure specific parameters of neutrophil and monocyte populations like mean and standard deviation (SD) of cell volume (MVI, SDVI), conductivity (MC1, SD1C), and light scatter (MS1, SD1S). These so-called positional parameters (PP) can detect morphologic changes in neutrophil and monocyte population. Using VCS parameters we investigated the correlation between megaloblastic neutrophils and monocytes in B12 and/or folate deficiencies. AIM The correlation of megaloblastic anaemia due to B12 and/or folate deficiencies with neutrophil and monocyte positional parameters. METHODS The study population included 427 patients. 177 had B12<145pg/mL and 189 patients had folate <2.33ng/mL. There were 61 cases with both serum folate and B12 deficiency (46 of them had normal ferritin, 31 had anaemia). Anaemia was defined according the WHO anaemia criteria (Hb<12 g/dL in women and Hb<13 g/dL in men). We collected blood samples from 45 healthy control subjects. The VCS parameters and full blood count was obtained by the Coulter LH 780 hematology analyzer. B12, folate and ferritin values were obtained using paramagnetic particle chemiluminescent immunoassay (Access2). P value less than 0.05 were considered significant.

The MV1 and SDVI of neutrophils and monocytes may be used for the detection of megaloblastic neutrophils and monocytes. Megaloblastic neutrophils and megaloblastic monocytes may be seen in B12 and/or folate deficiencies. Positional parameters have shown statistical role in the detection of those deficiencies in contradiction with the widely used MCV, because they are not affected by the presence at the same time of iron deficiency or other reasons of anaemia. Although plausible, this hypothesis needs to be sustained clinically by a prospective study.

EVALUATION OF AN IMPROVED MICROCUVETTE FOR FAST AND EASY POINT OF CARE HEMOGLOBIN DETERMINATION

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Background. Point of care testing for hemoglobin is often carried out using disposable microcuvettes based on the photometric determination of azidemethemoglobin [1]. The use of such microcuvettes enables a fast and accurate determination of hemoglobin outside the laboratory. One major problem arising from the use of microcuvettes is the formation of air bubbles during sample filling as it can cause incorrect hemoglobin readings. An improved microcuvette geometry comprising the integration of a slot which avoids this problem was developed by EKF-diagnostic GmbH (Hemo_Control microcuvette) [2]. Aims The aim of the study is the validation of the improved microcuvette on the Hemo_Control photometer in comparison to the classical reference method in hemoglobinometry and also to compare it to another widely-used microcuvette system (HemoCue B-Hemoglobin system). Methods. To evaluate the accuracy of the system, 112 venous blood samples (concentration range 50-256 g/L) were measured over 10 days with the Hemo_Control system, the Hemocue system and the reference method NCCLS [3]. 8 Hemo_Control devices, 19 Hemo_Control microcuvette lots and 4 operators were involved. The precision was calculated according to NCCLS EP5A [4]. Results. The results show an excellent correlation between the hemoglobin measurements from the Hemo_Control system and the NCCLS reference method for venous samples irrespective of the lot of microcuvettes. The correlation coefficient was 0.998 with an intercept of 0.005 g/dL; the standard error was 0.26 g/dL. This lot to lot precision means that recalibration of the system is unnecessary. Furthermore the correlation coefficient to the results of Hemocue B devices was 0.997 with an intercept of -0.46 g/dL. The standard error was 0.37 g/dL. The measured total precision of the Hemo_Control system was to 1.1%. Assessing 100 venous blood samples, the within-run-precision gave a CV of 0.63%. Summary/Conclusions The new microcuvettes show excellent accuracy and precision. The filling behaviour of the new microcuvette is considerably improved and the risk of air bubbles is substantially reduced. The measuring time is also shorter.
1585
URINARY HEPcidIN LEVEL AS AN EARLY PREDICTOR OF IRON DEFICIENCY IN CHILDREN

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Background: The ideal screening test would be capable of identifying iron deficiency in the absence of anemia. This would allow for the treatment of iron deficiency in the pre-anemic stage, preventing iron deficiency anemia and its associated mental, motor, and behavior effects. Such a test is not widely used at this time. Aim of the work: To detect role of urinary hepcidin level in early prediction of iron deficiency in children.

Methods: This case control study was performed on 100 children in Hematology Unit of Pediatric Department, Zagazig University Hospital, Egypt. Parental informed consent was obtained from all participating cases to be eligible for enrollment into the study. The medical ethics committee of the hospital approved the protocol. Children classified into 25 cases of iron deficiency stage one (iron depletion), 25 cases stage two (iron-deficient erythropoiesis), 25 cases stage three (iron deficiency anemia) and 25 healthy children as a control group. Estimations of iron parameters were done (iron, ferritin, transferrin, free protoporphyrin, total iron binding capacity and transferrin saturation). Urinary hepcidin 25 level was detected by competitive ELISA. 96-well plates were coated with anti-human hepcidin antibody and biotinylated hepcidin-25 as tracer. Analysis of selected data was done by statistical software (SPSS for Windows, version 11; SPSS; Chicago, IL). All values were given as mean ± SD. One-way analysis of variance (ANOVA) was used to assess differences among means of the study groups. Multiple regression analysis was used to assess the influence of multiple variables on a single variable. Receiver operating characteristic (ROC) curve analysis was used to determine the discriminative properties of various cutoff levels of urinary hepcidin Levels. A value of < 0.05 was considered significant. Results: Our results revealed that urinary hepcidin-25 levels were significantly lower in all stages of iron deficiency than in control group, more significant reduction in its level was observed with the progress in degree of iron deficiency and inflammation are often associated with and contribute to the impaired erythropoiesis in these patients. Aims: Evaluate the possible role of inflammation in the anemia and iron deficiency in various stages of renal failure. Methods: Adult patients with CKD and anemia who either undergoing dialysis or not undergoing transfusion and iron supplementation. Haematological parameters (Sysmex XE2100), iron status and inflammatory activity were carried out in 77 patients with CKD (26 Stage II, 36 Stage III and 15 Stage IV of impaired glomerular filtration), 57 normal individuals (CG), and 22 normal subjects with iron deficiency anemia without renal disorder (IDA). Results: 35 patients had anemia (RA) with iron deficiency, and 44 had functional iron deficiency (FID) (serum ferritin <100 µg/dL and/or transferrin saturation (TS) <20%). Comparing patients with RA with and without iron deficiency, it was observed that levels of protein C-reactive protein (CRP) higher in group with FID (p <0.0001, Mann-Whitney test) and higher levels of soluble transferrin receptor (sTfR) in group with FID (p< 0.0144). The degree of haemoglobinization of reticulocytes (Ret-He) was preserved in patients with CKD, similar to normal CG, both higher than IDA. A significant correlation (Spearman coefficient) between Ret-He and Mean Cellular volume (MCV) was observed in patients with CKD (r=0.6152, p= 0.0001), RA with FID (r= 0.6157, p< 0.0001), CG (r= 0.392, p= 0.0172) and IDA group (r= 0.4557, p= 0.04356). It was showed a progressive decreasing in hemoglobin level as renal dysfunction worsened. Although ferritin levels did not showed difference among the stages of DRC, levels of transferrin iron binding capacity (TIBC) and sTfR were increased and TS declined with increasing renal impairment. EPO levels were lower in the CKD than in RA with advanced activity (CRP levels) were higher in the group with AR and FID (p<0.0001) than RA and CG (p<0.0001). There was no correlation between haematological and iron parameters with CRP. Conclusions: According to the results we could conclude that the frequency of iron deficiency in those patients with CKD is quite high (57%), and that both anemia and inflammation are more important in patients with iron deficiency anemia without renal disorder. The role of inflammatory activity evaluated by CRP levels was significant.

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STUDY ON THE RESULTS OF ERYTHROPOIETIN TREATMENT IN CANCER PATIENTS FROM SOUTHERN TRANSYLVANIA

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Background: Erythropoietin reduces transfusion, improves quality of life and increases the response to treatment of patients with cancer. Aim: We aimed to study the efficacy and safety of β-epoetin in cancer patients with different types of cancers. Methods: Of the 624 patients with oncological and onco-hematological diseases, hospitalized in Emergency Clinical Hospital Sibiu, treated with β-epoetinum Sibiu in June 2009 - December 2010, all the 86 patients, who started therapy during this period were selected, in which we determined the serum hemoglobin initially and than at intervals of one month. β-epoetinum dose was 50,000 IU/week, and if the serum hemoglobin did not increase after one month at least with 1 g/dl, the dose was doubled. In patients with an average hemoglobin decrease after one month of treatment, the dose was reduced. The dose was also adjusted according to the type of cancer and they were statistically analysed. Results: The average age of the 86 patients was 65.36±13.33 years. Distribution by gender: women - 53, men - 33. At the start of treatment, mean serum hemoglobin was 9.32+/−1.20 g/dl. It grew up to 10.92+/−1.26 g/dl (p<0.00001) after the first month, 11.47+/−0.97 g/dl (p<0.00001) after 2nd month, 12.23+/−1.20 g/dl (p<0.00001) after 3rd month. After the first month of treatment, mean hemoglobinemia increased 1.6 g/dl, after 2 months - with 2.15 g/dl, and after 3 months - by 2.9 g/dl. The average monthly increases were observed in patients with chronic nonlymphoid hematological malignancies (1.95 g/dl, 2.66 g/dl, 3.97 g/dl), followed by those with chronic lymphoid hematological malignancies (1.86 g/dl, 1.66 g/dl, 2.79 g/dl). The smallest serum hemoglobin levels increases were observed in patients with solid neoplasms: after a month average increase of 1.17 g/dl, and 2 months - 2.09 g/dl. Most patients tolerated the treatment well. From the 624 patients treated with β-epoetinum made in the 16 months, one patient developed splenic infarction and thrombosis of the inferior vena cava (with a favorable evolution on anti-coagulant therapy), 1 - thrombophlebitis of a leg, 2 - alergodermia, 2 - high blood pressure and 5 - dyspeptic symptoms possibly related to the treatment. There were no recorded deaths related to the β-epoetinum therapy. Summary: β-epoetinum therapy had an efficent control of the anemia in the majority of the onco-hematological patients, reducing transfusion needs. The largest increases in serum hemoglobin were observed in patients with chronic nonlymphoid hematological malignancies, followed by chronic lymphoid hematological malignancies and those with solid malignancies. Side effects were rare and no deaths resulted.

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EVALUATION OF ANEMIA IN PATIENTS WITH CHRONIC KIDNEY DISEASE NOT SUBMITTED TO DIALYSIS TREATMENT

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Background: Anemia is a common condition in patients with chronic kidney disease (CKD) due to insufficient erythropoietin (EPO) production. Iron deficiency and inflammation are often associated with and contribute to the impaired erythropoiesis in these patients. Aims: Evaluate the possible role of inflammation in the anemia and iron deficiency in various stages of renal failure. Methods: Adult patients with CKD and anemia who either undergoing dialysis or not undergoing transfusion and iron supplementation. Haematological parameters were carried out in 77 patients with CKD (26 Stage II, 36 Stage III and 15 Stage IV of impaired glomerular filtration), 57 normal individuals (CG), and 22 normal subjects with iron deficiency anemia without renal disorder (IDA). Results: 35 patients had anemia (RA) with iron deficiency, and 44 had functional iron deficiency (FID) (serum ferritin <100 µg/dL and/or transferrin saturation (TS) <20%). Comparing patients with RA with and without iron deficiency, it was observed that levels of protein C-reactive protein (CRP) higher in group with FID (p <0.0001, Mann-Whitney test) and higher levels of soluble transferrin receptor (sTfR) in group with FID (p< 0.0144). The degree of haemoglobinization of reticulocytes (Ret-He) was preserved in patients with CKD, similar to normal CG, both higher than IDA. A significant correlation (Spearman coefficient) between Ret-He and Mean Cellular volume (MCV) was observed in RA group (p= 0.6152, p=0.0001), RA with FID (r= 0.6157, p< 0.0001), CG (r= 0.392, p= 0.0172) and IDA group (r= 0.4557, p= 0.04356). It was showed a progressive decreasing in hemoglobin level as renal dysfunction worsened. Although ferritin levels did not showed difference among the stages of DRC, levels of transferrin iron binding capacity (TIBC) and sTfR were increased and TS declined with increasing renal impairment. EPO levels were lower in the CKD than in RA with advanced activity (CRP levels) were higher in the group with AR and FID (p<0.0001) than RA and CG (p<0.0001). There was no correlation between haematological and iron parameters with CRP. Conclusions: According to the results we could conclude that the frequency of iron deficiency in those patients with CKD is quite high (57%), and that both anemia and inflammation are more important in patients with iron deficiency anemia without renal disorder. The role of inflammatory activity evaluated by CRP levels was significant.
in the physiopathology and progression of anemia was not observed, but other laboratory markers such as interleukins and hepcidin may provide evidence about that association. Diagnosis of iron deficiency in patients with CKD is not always sufficiently investigated. Deprivation of iron in patients not undergoing dialysis should be diagnosed and, if possible, corrected. An increase in hemoglobin levels may improve the quality of life of these patients.

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EFFICACY OF COMBINED ORAL DEFERIPRONE AND SUBCUTANEOUS DESFERRIOXAMINE IN IRON-OVERLOADED CHILDREN WITH BETA-THALASSEMAIA DISEASE: A TWO-YEAR EVALUATION

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Background. Iron overload is a serious complication found in transfusion-dependent patients with beta-thalassemia disease. Subcutaneous infusion of desferrioxamine (DFO) for five days per week creates the compliance and tolerability problem especially in pediatric patients.

Methods. The daily dose of 50 mg/kg increasing up to 75 mg/kg of Thailand locally-manufactured deferiprone (DFP) plus twice weekly subcutaneous infusion of DFO 40 mg/kg to 58 transfusion-dependent children with beta-thalassemia disease. Their mean age was 11.0 years (SD, 5.7).

Results. The patients had previously received regular transfusion for a mean duration of 9.1 years (SD, 3.4). All of them had been chelated by subcutaneous DFO for a median duration of 4.5 years (interquartile range 2.4-6.7) with an average dose of DFO at 27.7 mg/kg/d (interquartile range 22.3-31.8) for five days a week. The median serum ferritin level at the beginning of the study was 2909.4 ng/ml (interquartile range 2384.0-3708.8) while the mean transfusional iron load during the studied period was 0.31 mg/kg/d (SD, 0.07). None of the patients were hepatitis B carriers or had seropositive hepatitis C infection. All patients survived. The efficacy of combined therapy was evaluated in the 53 patients completed 24 month follow-up period. The median declination of serum ferritin was gradually increased: 149.6 ng/ml at 4 months, 404.4 ng/ml at 8 months, 663.7 ng/ml at 12 months, 746.7 ng/ml at 16 months, 772.3 ng/ml at 20 months and 1225.7 mg/ml at 24 months. Finally, the median serum ferritin level at 24 month was 1,458.7 ng/ml (interquartile range 1,004.6-3258.1). Eight out of 10 patients who had serum ferritin less than 1,000 ng/ml underwent cardiac MRI and LIC study. All patient had normal myocardial T2* with a median of 39.7 ms (interquartile range 33.3-43.4).

Conclusion. No patient experienced agranulocytosis. Conclusions. The preliminary study of using locally-manufactured DFP combined with a two-day infusion of DFO showed an effective and tolerable means in hematolobing-overloaded iron in transfusion-dependent children with beta-thalassemia disease.

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ACCURACY OF NONINVASIVE HEMOGLOBIN MEASUREMENT IN ANEMIC PATIENTS WITH KNOWN LOW HEMOGLOBIN LEVELS

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Background. Hematology/Oncology patients with known anemia (low hemoglobin) are subject to routine invasive blood sampling in order to the monitoring of hemoglobin levels and the determination of red blood cell transfusion. Aims. The intention of this study is to determine whether noninvasive hemoglobin readings (SpHb) from patients with low hemoglobin are accurate compared to invasive hemoglobin measurements (tHb). Methods. Eligible in/outpatients at the Japan Red Cross Medical Center diagnosed with hematological or oncological diseases were enrolled following informed consent. Both adult and pediatric patients, aged ranging 41 to 85 years, had hemoglobin measurements performed by venous blood draws analyzed on a Sysmex XE-2100 hematology analyzer. Concurrently, noninvasive SpHb spot check measurements were obtained with a Resposable finger sensor (R2-20 and R2-25, revision E) connected to a Radical-7 Pulse CO-Oximeter (SET software version 7.6.0.1) (Masimo Corp., Irvine, CA USA). Both adult and pediatric size sensors were used. SpHb values were compared to tHb by calculating bias and precision and with a Bland-Altman plot. If the internally calculated signal confidence indicator on the Radical-7 was less than 50%, then a SpHb value was not displayed on the device. Low signal confidence occurs due to low perfusion in the finger, excessive motion, or other confounding factors. Data pairs that included low signal confidence readings were excluded from analysis. Results. Fifty patients (36 male/15 female) with an average age of 66 years were enrolled. A total of 50 analyzed data pairs included tHb ranging from 6.1-13.1 g/dL (from Sysmex device) and noninvasive hemoglobin SpHb ranging from 6.4-14.6 g/dL. Data were separated based on sensor type/finger size (R2-20 vs. R2-25). For patients with smaller fingers using sensor R2-20, the bias and standard deviation were -0.31 ± 0.66 g/dL. For patients using the adult size sensor (R2-25) the bias and standard deviation were 0.62 ± 1.02 g/dL. A subset of subjects (n=8) with very low hemoglobin (<8 g/dL) showed a bias of 0.54 ± 0.23 g/dL. Conclusions. Based on the results of these 50 pairs, noninvasive SpHb with the Radical-7 showed good agreement with invasive tHb measurements from the laboratory reference device. Among patients with very low hemoglobin (<8 g/dL), SpHb showed even better agreement with the laboratory reference device with a bias and standard deviation of 0.84 ± 0.23 g/dL. The bias and standard deviation were consistent with a previous report of SpHb accuracy in volunteers undergoing hemodilution. Use of the Radical-7 with SpHb in this clinic allowed for instant, accurate and noninvasive hemoglobin monitoring in hematology/oncology patients, avoiding further blood loss in this already anemic population. This device is considered to be useful to determine the trigger point of red blood cell transfusion without venesection.
Background. Idiopathic pulmonary hemosiderosis (IPH) is a rare disease of unknown etiology, characterized by recurrent episodes of diffuse alveolar hemorrhage and sideropenic anemia which occurs most frequently in children. Sudden decrease in hemorrhage and hematocrit associated with the onset of active respiratory disease is strongly suggestive of IPH. Diagnosis of IPH should be considered when children have iron deficiency anemia and pulmonary signs and symptoms such as cough, hemoptysis and dyspnea. Materials and methods. We carried out a retrospective study using the medical charts of patients diagnosed with IPH, admitted at the Hematology-Oncology department - University Pediatric Clinic in Skopje which is the only institution that provides tertiary care and copes with this problem in our country. Results: Throughout a 45 years period (1965-2010) 45 patients (21 males and 22 females) were diagnosed with IPH, with an incidence of 0.38 per million yearly. Mean age at diagnosis was 6±3.64 years (range 0.25 to 15) with mean duration of follow up of 86±65. The diagnosis was established through the evidence of recurrent pulmonary hemorrhages, associated with severe repetitive mycrocytic hypochromic anemia, and was confirmed by the detection of hemosiderin-laden macrophages in Perl reaction from gastric washings or bronchoalveolar lavage. Open lung biopsy was made just in 1 patient. Initial treatment consisted of prednisone in 40 patients (93%). Ten patients (23.2%) required long-term corticosteroid because of recurrent attacks; 19 patients (44.18%) required other immunosuppressants (Immunar or Leucaran) in addition to prednisone to control their hemoptysis. Ten patients died (9 of massive pulmonary hemorrhage and 1 from pneumonia caused of Varicella) 1 to 12 years post diagnosis. Unfortunately, 23 patients were lost of evidence after mean follow up period of 50,0±49.08 months. Ten patients are alive: 2 with long term follow up of 35 and 27 years and being without treatment of 27 and 28 years respectively. Five year survival for IPH patients in our study was 78% (by Kaplan-Meier method). Conclusions. Pediatricians should raise suspicion for intrapulmonary bleeding in a patient who has recurrent dyspnea, hemoptysis and iron deficiency anemia. Our results show that long-term survival in IPH is possible with introducing early and long-term immunosuppressive therapy.
BACKGROUND: Cardiovascular Magnetic Resonance (CMR) allows an accurate and reproducible quantification of left ventricular (LV) parameters. In Thalassemia major (TM) patients different normal LV values have been reported due to chronic anemia and eventually pre-existing iron burdens. Moreover, in this population it is unknown the influence of sex and age on LV parameters and no ranges of normal have been reported using MASS® software. Aims. The aim of this study was to establish the ranges for normal LV volumes, mass and ejection fraction normalized to the influence of body surface area (BSA), age and sex from CMR in a large cohort of well-treated TM patients without myocardial iron overload. Materials. Among the 923 TM patients who underwent CMR within the MIOT network for the assessment of cardiac iron overload, function and fibrosis, we selected 142 patients with no known risk factors or history of cardiac disease, normal electrocardiogram, no myocardial fibrosis and no myocardial iron overload (all the cardiac segments with a normal T2* value).

METHODS: Moreover, we studied 71 healthy subjects matched for age and sex. LV function parameters were quantitatively evaluated in a standard way by SSFP cine images using MASS® software. LV end-diastolic volume, end-systolic volume, stroke volume, and mass were normalized to BSA (EDVI, ESVI, SVI, mass). Results. Table 1 shows the comparison of the CMR parameters with differentiation for sex and age in TM patients and healthy subjects and the cut-off of normality defined as mean - 2 standard deviation (SD). TM patients showed significantly lower BSA than the controls (P<0.0001). Significantly higher EDVI and SVI were found only for males <14 years and >30 years. Significantly higher LVEF were found only for males <14 years. In TM patients all LV volumes indexes were significantly larger in males than in females (P<0.0001 in all age groups). The EF was not different between the sexes. In males the ESVI and the EF were significantly different among the age groups (P=0.006 and P=0.001, respectively). In females no significant differences were detected among the age groups. Conclusion. In a large cohort of well-treated TM patients significant differences in LV parameters compared to controls were limited to males <14 years and >30 years. Appropriate "normal" reference ranges normalized to BSA, sex and age should be used to avoid misdiagnosis of cardiomyopathy in TM patients.

All patients had been regularly transfused and chelated since early childhood. Moreover, we studied 71 healthy subjects matched for age and sex. RV function parameters were quantitatively evaluated in a standard way by SSFP cine images using MASS® software. RV end-diastolic volume (EDV), end-systolic volume (ESV) and stroke volume (SV) were normalized by body surface area (EDVI, ESVI, SVI). Results. Table 1 shows the comparison of the CMR parameters with differentiation for sex and age in TM patients and healthy subjects and the cut-off of normality defined as mean - 2 standard deviation (SD). TM patients showed significantly lower BSA than the controls (P<0.0001). TM males (except age group 14-20 yrs) showed significantly higher RV EF compared to controls. In TM patients all LV volumes indexes were significantly larger in males than in females (P<0.0001 in all age groups). The EF was not different between the sexes. In males as well as in females the RV volumes were no significant different among the age groups, while in males the EF was significant different (P=0.004). Conclusion. In a large cohort of well-treated TM patients males showed significantly higher RV EF compared to controls. Appropriate "normal" reference ranges normalized to BSA, sex and age should be used to avoid misdiagnosis of cardiomyopathy in the clinical arena in TM patients.
subjects with thalassemia. Multiecho T2* MRI is a well-established technique for iron overload assessment in heart and liver but there are very few reports concerning the kidneys in both healthy subjects and transfused patients. Aims. Our aims were to assess the feasibility and reproducibility of the MRI technique for measuring kidneys T2* values, to establish the lower limit of normal in a cohort of healthy subjects and to correlate the obtained values with age and gender. Methods. Twenty healthy subjects (13 men and 7 women, mean age 29.1 ± 7.2 years) underwent MRI exam. One transverse slice through the kidneys was obtained by a T2* gradient-echo multiecho sequence. T2* measurement was performed with a previously validated software program (HIPPO-MIOT IFC-CNR®). For each kidney, T2* values were calculated in three different regions of interest. The ROI T2* values were averaged to obtain a representative value for both kidneys. The mean kidney T2* value was also calculated. The lower limit of normal for the T2* value was calculated on log-transformed data as mean minus 2 standard deviations. Results. Measurement of renal T2* values was feasible in all subjects. Average processing time was about 2 minutes. For the mean T2* value the coefficient of variability (CoV) and the intra-class coefficient correlation (ICC) were respectively 6% and 0.9 for the intra-operator reproducibility and respectively 12% and 0.8 for the inter-operator reproducibility. There was not a significant difference between left and right kidney T2* values (55.1 ± 8.1 ms vs 51.5 ± 9.0 ms, P = 0.183). The lower limit of normal for the mean kidney T2* value was 86 ms. The mean kidney T2* value did not show a significant difference between men and women (men 50.6 ± 8.9 ms vs women 55.6 ± 5.7 ms, P = 0.153). There was no correlation between mean kidney T2* value and age (r= -0.049, P=0.838). Conclusions. In conclusion, renal T2* measurements appear to be feasible, reproducible, non-time-consuming and reliable.

The percentage of patients who maintained a normal global heart T2* value (≥20 ms) was comparable between DFP-DFO (96%) versus DFP (100%) and DFO (98.1%) groups. Among the patients with myocardial iron overload (MIO) at baseline (global heart T2*<20 ms), in all three groups there was a significant improvement in the global heart T2* value (DFP-DFO: 4.2±3.9 ms P=0.004, DFP: +3.8±2.6 ms P=0.015 and DFO: 3.7±5.5 ms P=0.007; Figure 1) and a reduction in the number of pathological segments (DFP-DFO: -3.2±3.8 P=0.026, DFP: -6.0±3.6 ms P=0.031 and DFO: -2.9±3.7 ms P=0.001). In DFO-DFP and DFP groups there was a significant increment in the left ventricular ejection fraction (EF) (4.3±5.1% P=0.035 and 5.0±6.4% P=0.045, respectively) as well as in the right ventricular EF (6.7±6.6% P=0.017 and 6.2±3.7% P=0.001, respectively). The improvement in the global heart T2* and in biventricular function were not significantly different in DFO-DFP compared to the other groups. Among the patients with hepatic iron at baseline (T2*<9.2 ms), only in DFP group there was a significant improvement in the liver T2* value (2.0±3.5 ms P=0.010). Liver T2* changes were not significantly different in DFP-DFO versus the other groups. Conclusions. Prospectively we did not find significant differences on cardiac and hepatic iron or in cardiac function in TM patients treated with sequential DFP-DFO versus the TM patients treated with DFO or DFP in monotherapy.
**1582**

**INTRA- AND INTER-OPERATOR REPRODUCIBILITY IN THE ASSESSMENT OF CARDIAC AND HEPATIC T2⁺ VALUES USING 3T MRI SCANNERS**

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**Aims**

The aim of our multi-centre study was to assess prospectively in a large clinical setting the efficacy of the DFP+DFO versus DFP and DFO in TM patients by quantitative MR. Methods: Among the 1135 TM patients enrolled in the MIOT (Myocardial Iron Overload in Thalassemia) network we selected quantitatively MR. Aims. Among the patients with myocardial iron overload (MIO) at baseline who had been received one chelator alone between the 2 MR scans We evaluated prospectively the 51 patients treated with DFP+DFO versus the 39 patients treated with DFP and the 74 patients treated. Iron overload was measured by T2⁺ multiecho technique. Biventricular function parameters were quantitatively evaluated by cine images. Results. The dosages were: combined therapy DFP 61.9±24.3 mg/kg per 6.1±1.4 days/week and DFO 40.7±6.0 per 5.5±1.1 days/week; DFP 73±13 mg/kg per 6.1±1.4 days/week; DFO 40.7±6.5 per 5.4±0.93 days/week. Excellent/good levels of compliance were comparable in the DFP+DFO (90.2%) versus DFP (94.9%) and DFO (95.9%) groups. No data are available in literature about prospective comparisons between DFP+DFO versus DFP and DFO in monotherapy. The reproducibility of T2⁺ measurements at 3T was documented by several studies, but the reproducibility of T2⁺ measurements at 3T has never been investigated. A good reproducibility is vitally important for longitudinal follow-up of patients and also greatly reduces the sample size required for research. Aim: This study aimed to evaluate the reproducibility of T2⁺ measurements at 3T. Methods. 38 transfusion-dependent patients (22 males, 36 ± 7 years) underwent MRI for T2⁺ assessment at 3T. A single transverse slice through the liver was acquired using a T2⁺ GRE multislice sequence. The T2⁺ value was determined over a large ROI of standard dimension, chosen in a homogeneous area. Three parallel short-axis views of the left ventricle were obtained using T2⁺ GRE multislice sequence. The left ventricle was segmented into a 16-segments standardized model and the T2⁺ value on each segment was calculated as well as the global value and the mid-ventricular septum T2⁺ value. MRI image analysis was performed using a custom-written, previously validated software (HIPPO MIOT®, IFC-CNR). To assess the reproducibility of T2⁺ values, data related to 20 patients were randomly selected from the entire data set. To evaluate the intra-operator variability, the 20 images were blindly reanalysed by the same observer who analysed the entire data set after two weeks. To evaluate the inter-operator variability, the selected images were presented in random order to an other operator, who didn’t know the results obtained by the other one. The difference between two different analyses was evaluated by calculating the coefficient of variation (CoV) and the interclass correlation coefficient (ICC). The CoV was calculated as the ratio of the SD of the half mean square of the differences between the repeated values, to the general mean. The ICC was obtained from a two-way random effects model with measures of absolute agreement. An ICC ≥ 0.75 was considered excellent, between 0.40 and 0.75 good, and < 0.40 unsatisfactory. Results: The ICC was always obtained (>0.929).

**Table 1. Reproducibility data for T2⁺ values at 3T.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Reproducibility Data for T2⁺ Values at 3T</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFP+DFO</td>
<td>ICC: 0.929, CoV: &lt; 0.40</td>
</tr>
<tr>
<td>DFP</td>
<td>ICC: 0.929, CoV: &lt; 0.40</td>
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<tr>
<td>DFO</td>
<td>ICC: 0.929, CoV: &lt; 0.40</td>
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**Background**

Detection and monitoring of tissue iron overload represent the key clinical factor in the management of patients with iron loading anemias. The Magnetic Resonance Imaging (MRI) technique T2⁺ performed at 1.5T scanners is the most available noninvasive technique to assess hepatic and cardiac iron content. However, recently high field scanners, primarily 3T scanners, have been installed widely and are used in the clinical setting, bringing the need to assess the feasibility and reproducibility of T2⁺ measurements at 3T. Feasibility of T2⁺ measurements at 3T has never been investigated. A good reproducibility is vitally important for longitudinal follow-up of patients and also greatly reduces the sample size required for research.

**Table 1. Reproducibility data for T2⁺ values at 3T.**
Conclusions. The intra- and inter-operator reproducibility for T2* measurements at 3T were very good and comparable with these ones previously found for T2* measurements at 1.5T. As expected, the coefficient of variation for the global heart T2* was the smallest, due to a compensation of outliers that may lead to high variability in a single segment measurement.

1583 PREVALENCE SURVEY OF HAEMOGLOBINOPATHIES AND IRON DEFICIENCY AMONG NATIVES AND IMMIGRANTS PREGNANT WOMEN. ONE YEAR REFERRALS IN HAEMOGLOBINOPATHY PREVENTION IN NORTHERN GREECE

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Haemoglobinopathies constitute the most frequent monogenic disorders worldwide and Thalassaemias are the most frequent genetic disorders in Greece. The risk of giving birth to a thalassaemic child depends on the incidence of the thalassaemic gene in the population under study. The aim of the study was to determine the prevalence of haemoglobinopathies and iron deficiency in Greek pregnant women and in immigrant pregnant women and their haematological characteristics and epidemiological issues. The carrier identification was carried out by a standard scheme, which included: CBC and red cell indices using the Coulter ONYX, A Cation Exchange HPLC variant system (Bio-Rad, Variant B-Thalassemia Short Program), to determine HbA, HbA2 and HbF levels. The different abnormal structural haemoglobins were investigated by electrophoretic techniques both at alkaline pH on cellulose acetate and at acid pH on citrate agar. Sickling test and tests for HbH inclusion bodies were also performed. Haemoglobin A2 was also quantified by column micro chromatography and serum ferritin levels were measured by micro Elisa technique. 664 pregnant women were recruited in the study.

376 (56.6%) of them were native Greek women and 288 (43.4%) were immigrants. 396 (59.6%) were at the first trimester of pregnancy, 226 (34.4%) were examined at the second trimester and 56 (8.5%) at the third trimester. 353 of the pregnant women that were tested in the first trimester had hemoglobin levels more than 11 gr/dl. 37 had hemoglobin levels from 9 to 10.9 gr/dl and 6 had hemoglobin levels less than 9 gr/dl. The hemoglobin levels of pregnant women during the second trimester was more than 10.5 gr/dl in 199 women, from 9 to 10.4 gr/dl in 22, and less than 9 gr/dl in 5 of them. The hemoglobin levels of pregnant women in the third trimester was more than 11 gr/dl in 20 of them, from 9 to 10.9 gr/dl in 14 and less than 9 gr/dl in 4 of them. Iron deficiency with ferritin levels less than 12 ng/ml had 213 (32.1%) women, while 131 (19.7%) had 12-20 ng/ml, 272 (41%) of them had ferritin from 20 to 50 ng/ml and 48 (7.2%) had more than 50 ng/ml ferritin levels. Native pregnant women were found to have higher levels of ferritin in all trimesters and this was statistically significant p=0.000. We found that the number of the native pregnant women that are examined at the first trimester is 13% less compared with those of the immigrants. This is likely due to the fact that the immigrants have been in the country for a longer period.

1584 IS ANEMIA CORRECTION AFTER SPLENECTOMY DEPENDENT ON THE CLINICAL SEVERITY OF HEREDITARY SPHEROCYTOSIS PRIOR TO SPLEEN REMOVAL?

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Hereditary Spherocytosis (HS) ranges from asymptomatic to a life-threatening transfusion-dependent anemia. Splenectomy corrects the anemia, though the intrinsic erythrocyte membrane defect persists. Our aim was to study how the anemia, erythropoiesis and iron status were affected in HS patients after splenectomy, in accordance with the HS severity presented prior to splenectomy, to ascertain if patient’s improvement could be related to the clinical severity experienced before spleen removal. In 60 HS patients (43 splenectomized and 22 unsplenectomized) and 85 controls, we performed a basic hematological study, osmotic fragility (OF) test and reticulocyte count; determined the plasma levels of bilirubin, erythropoietin (EPO), soluble transferrin receptor (sTfR), folic acid, vitamin B12, iron, ferritin, and transferrin; and calculated reticulocyte production index (RPI). Our data showed that splenectomy lead to correction of the anemia (normal values of erythrocytes, hemoglobin and hematocrit), however mean cell hemoglobin concentration (MCHC) sustained its high value and OF was higher in splenectomized than in unsplenectomized patients; red cell distribution width (RDW), bilirubin, EPO, sTfR, reticulocytes, RPI, folic acid, vitamin B12, iron, ferritin, transferrin, were reduced in relation to unsplenectomized, though significantly higher than controls. In splenectomized patients erythrocytes, hemoglobin concentration and hematocrit presented a trend to decrease with worsening of HS (as classified prior to splenectomy) and bilirubin and OF a trend to increase. In summary, splenectomy lead to a correction in the anemia and this improvement seems related to the severity of HS prior to splenectomy.

Funding. This study was supported by a PhD grant (SFRH/BD/22442/2005) attributed to S.Rocha by FCT and FSE.

1585 INTRAVENOUS IRON MONOTHERAPY FOR THE TREATMENT OF NON-IRON DEFICIENCY ANEMIA IN CANCER PATIENTS UNDERGOING CHEMOTHERAPY

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Background. Anemia is a common complication of malignancy, occurring in over 50% of patients and may increase to more than 90% in patients with certain types of cancer and in those undergoing chemotherapy or radiation therapy. Anemia often enhances symptoms of fatigue, weakness, and dyspnea and thus, it may worsen Quality of life (QOL) and performance status in cancer patients. Blood transfusion is an effective treatment of anemia, but the effect is often temporary, and is linked to many serious adverse effects. Erythropoiesis-stimulating agents (ESA) produce significant increase in Hb level, decrease transfusion requirements, and improve QOL. However, 30-50% of patients do not respond. Additionally, many concerns were recently raised over the routine use of ESA to treat anemia in cancer patients; in several studies, ESAs were found to shorten overall survival or time to tumor progression. Aims: This trial will assess the efficacy and feasibility of IV iron therapy in cancer patients with non-iron deficiency anemia who are undergoing treatment with chemotherapy or radiation therapy without the use of ESAs. The study was approved by the Institutional Review Board (IRB) and written informed consent was obtained. Methods: We compared patients with solid cancers and non-iron deficiency anemia (hemoglobin (Hb) level < 11 g/dl, ferritin level > 100 ng/ml, transferrin saturation > 20%, with normal folate and vitamin B12 levels) who are receiving anti-cancer treatment with chemotherapy or radiation therapy or diet. Anemia related to hemolysis, bleeding, or bone marrow infiltrations were excluded. All patients received 200 mg of ferrous sucrose given in short intravenous (IV) infusion weekly for a total of 12 weeks without the use of ESAs, blood transfusion, or other supplements. Hb level was measured at baseline, every 3 weeks, and at week 14. Adverse events related to IV iron were prospectively reported. Results: During the study period, 19 patients (14 females, 5 males) were included. The mean age (+ SD) was 58 (+ 10.2) years. All patients had cancer and were on active chemotherapy; 8 patients withdrew from the study at week 1, 4 and 6; convenience was the only reason in all. These other patients were transfused while on iron therapy and considered as treatment failure. Thirteen patients had at least 8 weeks of IV iron therapy (10 had full 12 weeks of therapy). At week 12, the mean increase in Hb level was 1.6 g/dl and 1.4 g/dl in patients with non-small cell lung cancer and breast cancer, respectively. None of the patients required transfusion, all patients achieved the target Hb level, and there were no serious adverse events. Efficacy of IV iron therapy is comparable to that of ESAs. Efficacy and feasibility of IV iron therapy for treatment of non-iron deficiency anemia in cancer patients is comparable to that of ESAs.
weeks and 3 had 6 weeks). The mean Hb level at baseline was 9.6 g/dL. Two weeks following the last dose of 10V iron therapy, 9 (56.5%) patients had more than 1.0 g/m increase in their Hb while 5 (18.6%) others had <1.0 g/m increase. The mean Hb level following completion of treatment was 11.8 g/dL; an overall mean increase by 1.9 (0.8, 5.3). No therapy-related toxicities were reported among all patients. Conclusions. IV iron treatment alone is safe and can reduce blood transfusion requirements and improve quality of Hb levels in children with sickle cell disease undergoing anti-cancer therapy. This approach requires further randomized studies.

**1586**

THE RELATIONSHIP OF MEGALOBLASTIC ANEMIA AND THYROID FUNCTION DISORDERS BETWEEN GENDER AND MENOPAUSE

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**Background.** Megaloblastic anemia (MA) preferentially affects cells with high metabolic turnover over such as hematopoietic precursors and gastrointestinal epithelium. Ageing is associated with thyroid function abnormalities and cobalamin deficiency that are mostly caused by autoimmune disorders leading to production abnormalities and gastric mucosa destruction respectively. **Aims.** In our study, we want to evaluate the relationship of MA and thyroid function disorders to sex and menopause. **Methods.** 157 patients (64 male, 93 female) who were deficient of either cobalamin and/or folic acid and had thyroid function abnormalities included in the study. **Results.** Age, diet habits, smoking, medical history of all and menopause status of women patients were recorded. Hemogram, reticulocyte count, peripheral smear, cobalamin, folic acid, ferritin, serum iron, total iron binding capacity, homocysteine, glucose, urea, creatinin, lipid profile, LDH, total bilirubin, AST, ALT, ALK, GGT, creatinin, TSH, anti-thyroglobulin, anti-thyroidperoxidase antibodies, anti-microsomal antibody, PT and APTT of all patients were measured. FSH was sampled from women who had menopause. ECG and PA Chest X-Ray were evaluated in each patient. Patients who had been diagnosed as megaloblastic anemia, thyroid function abnormalities, chronic renal failure, pregnant or lactation, coronary artery disease, diabetes mellitus, chronic liver disease were excluded from the study. Data were analysed by SPSS 13 for Windows. Pearson ki-square and Student’s t test were used for statistical study. **Results.** 93 (59.2%) cases were women and 64 (40.8%) cases were men. 124 (79%) cases had only MA and 35 (20%) cases had MA with thyroid dysfunction. MA only group had more women than men and more premenopausal women then post-menopausal one but these were not statistically significant (p>0.05). 19 (20.5%) of women had MA and thyroid dysfunction and 52.6% of cases were in menopause and 47.4% were not. MA were more common in women having menstrual cycle, but this has not reached statistical significance (p>0.05). The peak incidence of all cases was between 40-49 years old and mean age was 44.6 ± 15.4. In only MA group, 50 (40.3%) of cases were under 40 years whereas 74 (59.7%) of cases were over 40. MA was significantly higher in patients over 40 (p= 0.042). 33 cases were found in MA and thyroid dysfunction group; 7 (21.2 %) of them were under 40 years old and 26 (78.8%) were over 40 and this was statistically significant (p=0.042). **Conclusions.** As a result, it was thought that regardless of sex and menopause status, all people after 45 years old should be examined for MA and thyroid dysfunction. It will be beneficial to make larger population studies in this field.

**1587**

FLOW CYTOMETRIC DETECTION OF PNH-LIKE CLONES ON PERIPHERAL BLOOD GRANULOCYTES IN PATIENTS WITH APLASTIC ANAEMIA IN PAEDIATRIC AGE GROUP

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**Background.** Aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH) are both clonal bone marrow stem cell disorders with closely interlinked pathophysiology. Bone marrow failure has been regarded as one of the triad of clinical manifestations of PNH and in turn has been described to evolve in patients recovering from aplastic anemia. Several studies investigated the link between PNH and AA, but very few tested this link in pediatric age group. Subjects and Methods: In this study, the presence of CD55 and/or CD59 defects (PNH-like clones) peripheral blood granulocytes was evaluated in 30 newly diagnosed children with AA and in 20 healthy matched controls using flow cytometric immunophenotyping. Results: Minor population with deficiency of both CD55 and CD59 was detected in 8 cases (26.7%), while CD55 deficiency was detected in 4% of cases (13.3%) and CD59 deficiency in 5% of cases (16.7%). Conclusion: The results of this study highlight the strong association between AA and PNH. Expansion or regression of these PNH-like clones in response to therapy needs more evaluation in relation to disease outcome and response to immunosuppressive therapy.

**1588**

CHANGES IN PLASMA LEVELS OF ADAMTS13 IN PAINFUL CRISIS AND ASYMPTOMATIC SICKLE CELL ANAEMIA

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**Background.** Sickle cell disease is a congenital disease with severe haemolytic anemia. This clinical statement is characterized with ischemic changes as a result of obstruction of vascular system by sickling erythrocytes. ADAMTS13 (A Disintegrin and Metalloprotease with Thrombospondin domain type 1 repeats) is a metalloproteinase that cleaves von Willebrand factor (vWF) multimers in plasma. Deficiency of ADAMTS 13 is the major pathogenic factor in thrombotic thrombocytopenic purpura (TTP). In some diseases in which thrombosis incidence is increased (ischemic stroke, ischemic heart disease, malignancies, collagen vascular disease, active infections, acute inflammation, regardless of etiology), the patients using clopidogrel, ticlopidine, glycoprotein Ib/IIa antagonist and pregnant women in which the levels of vWF and ADAMTS-13 can be affected were excluded from the study. The ADAMTS-13 and vWF antigen plasma levels were determined by ELISA method quantitatively. **Results.** The levels of vWF of the patient groups were found significantly higher than the levels of the control groups (p=0.0001). There was no statistically difference when the ADAMTS-13 levels of both 2 groups were compared with the levels of the control group (p=0.295 and p=0.062). The ADAMTS-13 / vWF ratio was statistically significantly lower in both painful crisis and asymptomatic period when compared with the control group (p=0.0001). **Summary.** While there is no relationship detected between ADAMTS 13 and SCA, the high levels of vWF might be an indicator of vascular disease in patients with SCA.

**1589**

ORAL IRON CHELATION CHALLENGES IN CHILDREN

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**Background.** The availability of oral iron chelation therapy signifies a notable change in clinical practice that has relieved patients undergoing chronic blood transfusion from the burden of desferrioxamine infusions. Despite this, getting children on oral iron chelation therapy has been a challenge. Notably, oral chelation therapy is particularly challenging for clinicians and parents. **Aims.** The aim of this case review is to present some of the challenges faced by doctors who deal with iron overload and oral chelation therapy in children. Making few suggestions and recommendation was another goal. **Method.** This was a case review analysis in which two sisters, five and seven years old, suffering from b-Thalassemia major and undergoing regular blood transfusion were monitored closely to follow their response to deferasirox, an oral iron chelator. This was in a form of file review as well as regular meeting with patients and parents. **Results.** These two sisters shared the same genetic background, diet, motivational tool (star chart), dose of deferasirox, and followed up at the same center to receive the same volume of blood per kg every three weeks. In spite of having all these similarities, they responded differently and the serum ferritin dropped smoothly to below 1000ng/ml for the seven years old sister while it remained above 2000ng/ml for the five
years old sister. For the younger girl (5-year old) it was reported by parents that it was difficult to get her to drink the full amount of the medicine.

We observed that low body weight for the girl made it hard to maintain the ideal deferasirox dose as any increment using the available tablet strength causes significant change in dose per kilogram. Producing lower tablet strength may help make dose adjustment easier in children. High deferasirox clearance in children between two to six years was reported in early clinical studies and could be contributing to the younger girl slow response. This has raised the question of the benefit of dividing deferasirox dose in children below five years to get a better control. Summary. Oral iron chelation therapy carries its own challenges in children on chronic blood transfusion. Children are resistant to drink the full amount of deferasirox and adjusting the dose utilizing the available tablets’ strength has been challenging. Increased deferasirox clearance in children below five years may raise the question of the benefit of dividing deferasirox dose to get to a better control. The use of star chart as a motivational tool is old but still found to be effective in improving medication compliance among our chronically transfused young patients. Observing the individuality of each patient is warranted as each patient is unique and therapy need to be tailored to suit him.

1590 CLINICAL OUTCOME OF PATIENTS WITH MYELOPHILOSIS ANEMIA ARISING FROM ADVANCED GASTRIC CANCER

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Background. Although it is not common to encounter patients with myelophihic anemia arising from advanced gastric cancer, clinical features and optimal treatment are not yet to be elucidated. Prognosis for gastric cancer patients with bone marrow metastases is extremely poor. The current study was performed to evaluate clinical outcome of patients with myelophihic anemia arising from advanced gastric cancer. Methods We retrospectively reviewed the medical records of 26 advanced gastric cancer patients with bone marrow metastases between September 1986 and February 2009 at Soonchunhyang University Hospital. Results The median age was 46 years (range 24-61 years). All patients had poorly differentiated adenocarcinoma, including 17 signet ring cell carcinomas. The majority of the patients showed thrombocytopenia, anemia, and elevation of lactate dehydrogenase. Sixteen patients (61.5%) were received palliative chemotherapy with a median of 4 cycles. (range, 1-13 cycles). Median survival durations after bone marrow metastases for entire patients were 37 days (95% CI, 12.5-61.5 days). The median survival times from bone marrow involvement were 11 days in the best supportive care group (range 9.5-12.5 days ) and 121 days (range 94.7-147.3 days ) in the palliative chemotherapy group (p <0.001). Patients died of tumor progression (11 patients, 45%), brain hemorrhage (6 patients, 23%), infection (5 patients, 21%), and DIC (1 patient, 4%). There were no chemotherapy related deaths. Conclusions. It is difficult to decide whether to proceed with aggressive treatment for gastric cancer patients with bone marrow metastases because of the hematologic findings, e.g. anemia, thrombocytopenia, and DIC. However, this study suggests that palliative chemotherapy should be actively considered in patients with myelophihic anemia arising from advanced gastric cancer.

1591 PULMONARY HYPERTENSION IN SICKLE CELL DISEASE: STUDY OF 137 PATIENTS RANDOMLY SELECTED FROM A PUBLIC HEMATOLOGY HOSPITAL IN RIO DE JANEIRO, BRAZIL

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Background. Sickle cell disease (SCD) is the most common monogenet- ic inherited hemoglobin disorders worldwide. Pulmonary Hypertension (PH) is one of the leading causes of morbidity and mortality and, in the other hand, hematologic disorders are potentially among the most common causes of pulmonary hypertension. The pathogenesis of pulmonary hypertension in hemolytic disorders is multifactorial, including hemoly- sis, impaired nitric oxide bioavailability, chronic hypoxemia, chronic vasoconstrictive episodes, chronic liver disease, and asplenia. Methods. We randomly select 150 patients who have been assisted in the emergency room of Instituto Estadual de Hematologia Arthur de Siqueira Cavalcan- ti (EMORIO), a public hematolog hospital of State of Rio de Janeiro, Brazil, from 2009 June to 2010 August. Thirteen was excluded from the analysis, ten who did not show up to echocardiography, two who suf- fer at least one episode of acute chest syndrome (one of the exclude cri- terion) and one who die. Patients signed the informed consent to partic- ipate. Results. The majority was SS genotype (90%). The mean age was 30.9 ranging from 13 to 60 years old. The male/female ratio was 1.6, 62% of male and 38% of female. PH was diagnosed by echocardiogram. We consider PH when tricusupid regurgient jet velocity (TRV) > 2.5m/s which correspond to a pulmonary artery systolic pressure of approximately 30-39 mm Hg. 27% was classified as having mild PH (TRV between 2.5 and 2.9m/s), 51% as having moderate to severe PH (TRV > 3.0m/s). We could not classify 3 patients because they have not TRV despite they have others signs of PH. There was no significant difference between genders. A logistic regression was performed to estimate the effect of lactate dehydrogenase (LDH), the use of Hydroxyurea (HU) and the age on the prognosis of PH. Aging is related to increase in the incidence of PH (OR=1.05 CI95%=1.01 to1.09). Elevation of the plasma LDH is followed by a little increase of the incidence in PH and should not be a predictor in this sample. HU confers protection (OR = 0.83 IC95% = 0.12 to 0.81). In contrast to patients with traditional forms of pulmonary arterial hypertension, patients with hemolytic disorders have a mild-to-moderate degree of elevation in mean pulmonary pressures, with mild eleva- tions in pulmonary vascular resistance. Conclusions. The hemodynamic etiology of pulmonary hypertension in these patients is multifactorial and includes pulmonary arterial hypertension, pulmonary venous hyper- tension, and pulmonary hypertension secondary to a hyper dynamic state. Currently, there are limited data on the effects of any specific treat- ment modality for pulmonary hypertension in patients with hemolytic disorders. This data suggest that HU could improve the outcome of SCD patients with PH.

1592 COMPARISON OF HBA2 QUANTITATION BY HPLC AND CE TO DETERMINE THE SEVERITY OF BETA GLOBIN GENE MUTATION IN THALASSAEMIA TRAIT

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Background. Thalassaemia is a group of inherited disorders of haemoglobin, characterised by a significant decrease in the rate of synthesis of one or more globin chains, which in beta thalassaemia will be the beta chain. In adults the haemoglobin A2 (HbA2) comprises 2-3.5% of total haemoglobin. In some patients the proportion of HbA2 is raised. This is diagnostic for beta thalassaemia trait, but can also be seen in some unstable haemoglobins. Mutations which cause decreased beta-globin gene expression are classified as either "beta-" or "delta-beta-" (reduced level of beta-globin synthesis) or beta (no beta-globin synthesis). Aim In this study the HbA2 was quantified using two different methods, capillary zone electrophoresis (CE) and high-pressure liquid chromatography (HPLC). The results were correlated with the mutations found via beta globin gene sequencing in order to establish whether the quantitation of HbA2 by either method can be used to determine the beta-globin mutation type (beta+ or beta0). Method The CE, HPLC and beta globin gene sequencing were used to establish a total of 31 subjects with HbA2 > 3.5% (indicative of beta thalassaemia trait). Results The HbA2 results produced by CE were statistically significantly lower than those obtained by HPLC when compared using a paired t-test (p<0.001). Three of the thirty one patients were found not to have any common beta globin gene mutations, but were included as the HPLC showed an HbA2 percentage of 3.6, 3.7 and 3.9 respectively. Of the 31 patients, 8 cases were diagnosed as having heterozygous beta0 mutations including 4 patients with codons 41/42 (T-TCT) mutations, 2 patients with codons 6/9 (+C) mutations, 1 patient with a codon 15 (+T) mutation and 1 patient with a codon 39 (CAG-TAG) mutation. Twenty patients were found to be heterozygous for beta+ mutations including 6 patients with IVS-1-5 (G-C) mutations, 6 patients with IVS-1-110 (G-A) mutations, 5 patients with -29 (A-G) mutations, 2 patients with -88 (C-T) mutations and 1 patient with an IVS-1-6 (T-C) mutation. When compared using the t-test there was no difference between the HbA2 results obtained using HPLC for the beta+ and beta0 mutations cohorts (p=0.06). The same comparison performed using the HbA2 results obtained using CE showed a statistically significant difference between the beta+ and beta0 mutation cohorts (p=0.02). The predictive values of the measurement of HbA2 in establishing whether a patient has a beta+ or beta0 mutation calculated using Receiver operating characteristic (ROC) curves are 0.701 and 0.763 for HPLC and CE respectively. Summary/Conclusion. From the results of this study the CE is more sensitive in distinguishing between beta+ and beta0 mutations. Although this difference is relatively modest it is suggestive of a trend which may be more profound if the cohort size was larger. The HbA2 level was significantly lower with the CE analyser. No attempt was made to compare the different beta+ and beta0 mutations as the sample size was insufficient.

1593
MOLECULAR CHARACTERIZATION OF BETA-THALASSEMA AND HEMOGLOBIN VARIANTS IN THE CZECH AND SLOVAK POPULATIONS: AN UPDATE
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Background. Beta-thalassaemia is considered a rare disorder in Middle Europe. Similarly to other non-malaria regions, the prevalence of beta-thalassaemia in Middle Europe reflects the historical as well as recent immigration and demographic changes that have influenced the genetic variability of the current populations living in this area. Aims. To assess the frequency and spectrum of mutations on the beta-globin gene in Czech and Slovak patients with clinical symptoms of beta-thalassaemia or beta-thalassaemia. The results of the initial part of this research were published almost two decades ago (Indrak et al., Hum Genet 1992; 88: 399-404) and the aim of this work was to update this original report. Patients and Methods: Nearly 380 cases from seven hematological centers of Czech and Slovak Republics were analyzed. Blood samples were available for blood cell count measurements, hemoglobin (Hb) electrophoresis, chromatography and cDNA analysis in beta-globin (beta+), beta2-thalassaemia mutations were identified in more than 260 hemoglobin variants from 152 unrelated families of Czech or Slovak descent. Most of the mutations were of Mediterranean origin and accounted for 70% of patients. New- ly discovered insertion of transposable element L1 into the beta-globin gene represents a novel etiology of beta-thalassaemia due to a silencing effect of the transposable element. The list of abnormal hemoglobins now contains 14 beta-globin variants, including the rare high oxygen affinity Hb Olomouc associat-
ed with familiar polycythemia and two unique Heinz body hemolytic variants (unstable Hb Hradec Kralove and Hb Hana). Conclu-
sions. In the Czech and Slovak populations, beta-thalassaemia appears to be an uncommon disorder, which, however, must be considered as the prevailing cause of congenital hypochromic macrocytic anaemia. All but four studied patients were heterozygous carriers, manifesting thalassaemia minor, with rare exceptions of dominantly inherited beta-thalassaemia with phenotypes that ranged from severe thalassaemia minor to thalassaemia intermedia. Three of the four homozygous or double-heterozygous beta-thalassaemia patients were recent immigrants from malaria countries. Genetic drift and migration in the past as well as recent immigration are responsible for introduction of the Mediterranean alleles, while several mutations, described in single families, originated locally.

1594
GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY A SINGLE INSTITUTION EXPERIENCE
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Background. Glucose-6-phosphate dehydrogenase deficiency (G-6PD) is an X-linked disorder affecting red cell metabolism. Its distribution varies significantly among different geographic regions. In Greece where this disease is endemic, an estimated 225,000 males and 92,000 females are affected. The main clinical manifestations are acute hemolytic anemia and jaundice triggered by infection or ingestion of fava beans or oxidotive drugs. Aims. To present data indicating the frequency of G6PD deficiency in the population admitted to the hospital of Chania city (Crete island) where there is extensive consumption of fresh or dried fava beans and previously have been reported several cases of favism Methods: During 13-year period (1997-2010), tests for the quantitative measurement of G-6PD activity by enzymatic colorimetric assay by a commercial kit (Trinity biotech, Menarini) were conducted on 1397 samples. Any individual with an activity level below 6,6 U/LHb and 146 (U/1012 RB) was considered G-6PD deficient. Results: Of the 1397 (1030 males, 367 females) screened, 267 (147 males, 120 females) were children. Among children 14/267 (5.24%) were immigrants and 32/267 (11.98%) were found to have G-6PD deficiency. Complete enzyme deficiency was shown in 19/267 (7.11%) males whereas 2/267 (0.74%) were immigrants. Moderate enzyme deficiency was identified in 13/267 (4.86%) females. 4/267 (1.49%) children were admitted to hospital with clinical manifestation related acute hemolysis. The children with hemolysis were males (2/5 were immigrants), younger than 5 years old and have consumed fava beans. Of the adults (220 males, 910 females) 65/1130 (5.75%) were deficient in G6PD. Complete enzyme deficiency was shown in 40/1130 (3.53%) males and 6/1130 (0.53%) were females. Moderate enzyme deficiency was identified in 19/1130 (1.68%) females. The overall incidence of the deficiency in screened population (97/1397) was 6.94%. The rate among men was 59/1030 (5.75%) and among females, 38/367 (10.35%), with the male to female ratio 1:2. Conclusion: G6PD deficiency seems to affect more females than males. As prenatal screening for G6PD is long established in Greece, clinical cases of favism are observed rarely. Acute hemolysis was found only in young children. We believe that the screening with a comprehensive education program should be performed for young children in order to prevent the occurrence of hemolysis.

1595
THE ROLE OF REGULATORY T CELLS AND FOXP3 EXPRESSION IN GREEK B-THALASSEMA MAJOR PATIENTS
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Background: The suppressive/immunomodulatory function of CD4+CD25+FoxP3+ regulatory T (Treg) cells is crucial for the maintain-
ce of immune homeostasis. Although a large number of studies have been performed on immune dysregulation with respect to beta-thalas-
seaemia major, including T and lymphocyte subsets, little information is available regarding the role of Treg cells in this patient group. Aim: The aim of the present study was to determine B cells and T cells subpopulations,
including CD4+CD25bright+Foxp3+ Treg cells, and to correlate possible findings to iron chelation therapy. Methods: Fifty-two patients with beta-thalassemia major (30 males, 22 females) aged 6.5-46 years (mean age: 24.14 ± 10.439 years) participated in the study. Patients were regularly transfused and were on a regular chelation program. Data recorded included age, sex, chelation regimen, iron overload as measured by conventional iron chelation. FEVER-TMRI, and liver T2MR, and laboratory function as measured by left ventricle ejection fraction (LVEF) and history of splenectomy. Iron chelation therapy consisted of deferoxasior in 22 patients, deferiprone in 12, deferoxamine in 9 and combination of deferoxamine and deferiprone in 9 patients. Patients with HIV, HVB, HCV and CMV infections were excluded from the study. Twenty-seven healthy children and adults of the same age served as controls. All peripheral blood samples were analyzed on FC 500 Flow Cytometer (Beckman Coulter). Four-color cytometric analysis was performed for the detection of CD19+ B cells, CD3+ T cells and T cells subpopulations CD4+CD4+, CD3+ CD8+, CD4+CD45RA+, CD4+CD45RO+, CD4+CD25+, CD4+ CD25bright+ Foxp3+ Treg using specific fluorochrome-conjugated monoclonal antibodies. The percentages of Treg were determined using the anti-CD4, anti-CD25, anti-CD127 (Beckman Coulter) surface and anti-FoxP3 (PCH101, e-Bioscience) intracellular monoclonal antibodies. Results: Percentages of CD19+ and CD4+CD45RO+ memory T cells were found to be significantly higher in patients compared to the control group (CD19+: 14.07±4.14 vs 7.11±2.26 and CD4+CD45RO+: 24.53±8.69 vs 16.38±4.01, p=0.0012 and p<0.0001, respectively). On the contrary, percentages of CD3+ and CD4+CD45RA naive T cells were significantly lower in patient cohort compared to controls (CD3+: 70.42±6.51 vs 74.57±6.28 and CD4+CD45RA: 17.64±6.24 vs 25.26±5.51, p=0.0099 and p=0.01, respectively). Finally, Treg cells were found to be significantly higher in patients compared to the control group (2.77 ±1.05 vs 1.75±0.57, p<0.0001). However, there were no correlation between Treg cells and any of the parameters studied (age, sex, iron chelation regimen, LVEF, serum ferritin, liver or heart MRI, history of splenectomy) (p>0.05). Conclusions: Elevated CD19 B cells as well as CD4+CD45RO memory T cells in thalassemia patients compared to controls might be indicative of chronic antigenic challenge, as a result of repeated blood transfusions. The increase of Treg percentage in patients could be attributed to their immune compensatory function. Further studies are needed in order to investigate the exact role of Treg in the immune system of thalassemia patients, as well as their potential in inducing transfusion tolerance.

1596 RETICULOCYTE HEMOGLOBIN AS AN INDICATOR OF IRON DEFICIENCY IN PATIENTS WITH CHRONIC KIDNEY DISEASE

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Background. The evaluation of iron status in patients with chronic kidney disease is crucial as it provides prerequisite information for deciding recombinant human erythropoietin treatment. As there are cases (e.g. inflammation) when the traditional biochemical markers for the estimation of iron deficiency seem to be inadequate, the introduction of more reliable tools remains a laboratory challenge. Reticulocyte hemoglobin equivalent (RET-He) is a new parameter provided by modern automated hematologic analysers as a component of complete blood count. It provides an indirect measure of iron availability for new red blood cell production and is considered an indicator of iron-deficiency erythropoiesis. Aims. To investigate the levels of RET-He in patients with chronic kidney disease, its correlation with other parameters of iron status and red blood cell indices and to examine whether it can be used as a useful marker in the assessment of iron status. Methods: A total of 31 patients and 18 controls were included in the study. Reticulocyte and red blood cell indices were measured with Modular P800 analyser (ROCHE). Results: The mean RET-He value was 25 ± 10 μmol/l (range 974-13166) before starting DFX treatment and was 1875 μmol/l (range 563-6845) at the time of study. Apart from the demographic and clinical data collection, serum immunoglobulins, IgG subclasses, serum levels of complement factors C3 and C4, and lymphocyte subsets were studied. Results: There were 5 males and 12 females with a median age of 26 years (range 15-32 years). Median serum ferritin level was 2258 μmol/l (range 974-13166) before starting DFX treatment and was 1785 μmol/l (range 563-6845) at the time of study. Serum ferritin decreased in 12 patients, increased in 5 and was stable in 2 patients. Fourteen patients were splenectomised. Serum IgG levels were increased in 7 patients, while IgA and IgM levels were increased in 4 and 2 patients respectively. Low C4 levels were found in 9 patients and low C3 in 2 patients. IgG subclasses were normal in most of the patients except IgG-1 levels, which were increased in 4 patients. Absolute total B and T lymphocytes were increased in 14 patients each as compared to the normal range. CD4+ and CD8+ cells were increased in 13 and 12 patients respectively. NK cells were also increased in 11 patients. CD4/CD8 ratio was increased in 8 patients, decreased in 2 patients and normal in 7 patients. Conclusions: Elevated CD19 B cells and CD4+CD25+ FOXP3+ Treg using specific fluorochrome-conjugated monoclonal antibodies.

1597 IMMUNOLOGICAL EVALUATION OF ?-THALASSEMIA MAJOR PATIENTS RECEIVING ORAL IRON CHELATOR DEFERASIROX

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Background. Several immunological abnormalities have been described in patients with beta thalassemia major. Deferasirox (DFX), an oral chelating agent used in the treatment of beta thalassemia major, has interesting properties including antiproliferative, apoptotic and antitumour effects. Aim: This study was performed to investigate whether DFX further contributes to the altered immune function in beta thalassemia major patients. Patients and methods. We prospectively studied the immune function in 17 consecutive beta thalassemia major patients between January and December 2009 at King Khalid University Hospital, Riyadh. All the patients were receiving regular blood transfusions from an early age and had previously received deferoxamine for iron chelation. The dose of DFX ranged from 20-30 mg/kg and the mean duration of DFX treatment was 24 months at the time of sample collection. Apart from the demographic and clinical data collection, serum immunoglobulins, IgG subclasses, serum levels of complement factors C3 and C4, and lymphocyte subsets were studied. Results: There were 5 males and 12 females with a median age of 26 years (range 15-32 years). Median serum ferritin level was 2258 μmol/l (range 974-13166) before starting DFX treatment and was 1785 μmol/l (range 563-6845) at the time of study. Serum ferritin decreased in 12 patients, increased in 5 and was stable in 2 patients. Fourteen patients were splenectomised. Serum IgG levels were increased in 7 patients, while IgA and IgM levels were increased in 4 and 2 patients respectively. Low C4 levels were found in 9 patients and low C3 in 2 patients. IgG subclasses were normal in most of the patients except IgG-1 levels, which were increased in 4 patients. Absolute total B and T lymphocytes were increased in 14 patients each as compared to the normal range. CD4+ and CD8+ cells were increased in 13 and 12 patients respectively. NK cells were also increased in 11 patients. CD4/CD8 ratio was increased in 8 patients, decreased in 2 patients and normal in 7 patients. Conclusions: The immunological changes observed appear to be nonspecific and previously described in thalassemia major patients, and unlikely to be contributed by DFX. Larger studies including other aspects of immune system are needed to understand the effect of DFX on immune function of thalassemia major patients including comparison of immune status before and after starting DFX.

1598 HYDROPS FETALIS IN A PK-DEFICIENT PATIENT HOMOZYGOUS FOR A PKL MISSENSE MUTATION IN CIS WITH A NOVEL PROMOTER NUCLEOTIDE SUBSTITUTION

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Background. Pyruvate kinase (PK) deficiency is a congenital disease with heterogeneous severity, ranging from a mild asymptomatic to a severe transfusion-dependent haemolytic anaemia. Rare cases of hydrops fetalis and death in the neonatal period have been reported. Aims: To report the molecular background of a PK-deficient patient with hydrops fetalis and severe transfusion-dependent chronic haemolytic anaemia. Clinical history: A 6 year old boy, of Indian origin, with a transfusion dependent chronic haemolytic anaemia. He was born with a non-immune hydrops fetalis and severe

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neonatal haemolytic anaemia requiring exchange transfusion: Hb=5 g/dL, reticulocytes=311 x 10^6/L and erythroblasts=250-100/leucocytes. His parents are non-consangunous and have no history of anaemia. Father's red cells PK activity was 6.4 IU/gHb and mother's was 5.5 IU/gHb (n.r. 8.4-15.2). Methods: Haematological parameters and PK enzyme activity were measured by standard procedures. After informed consent, genomic DNA was extracted from EDTA peripheral blood samples and PKLR gene was studied by PCR-sequencing. Results: We identified a previously described PKLR gene missense mutation, c.1220A>G (p.Glu407Gly), in exon 9 at the homozygous state. Additionally, a second nucleotide substitution in the promoter region, -119G>A, not previously described, was also found at the homozygous state. Father and mother are heterozygous for both mutations. Conclusions: We describe a patient of Indian origin with a PK deficiency anaemia, homozygous for the missense mutation c.1220A>G (p.Glu407Gly) in cis with the nucleotide substitution -119G>A, a new promoter mutation. Mutation p.Glu407Gly was previously described in an Indian patient with severe haemolytic anaemia at birth, requiring exchange transfusion. The non-conservative substitution of the highly conserved Glu407 acidic residue for the tiny and small residue Gly occurs in the PK-R subunit A domain (A68a), near the active site of the enzyme, which is located between the B and A domains. Nevertheless, the second mutation identified in PKLR promoter region, -119G>A, modifies the CAC/Sp1 motif (-119gggtgg-113), and is expected to decrease the gene transcriptional activity. The additive effect of these two mutations may explain the severity of the phenotype.

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RELATIONSHIP BETWEEN HAPTOGLOBIN GENOTYPE, INTERLEUKINS AND CLINICAL FINDINGS IN BRAZILIAN SICKLE CELL ANAEMIA PATIENTS

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Background: Oxidative stress, particularly in the endothelium, exerts a strong influence on the genesis of sickle cell anaemia (SCA) vaso-occlusion and, consequently, on patients’ clinical evolution and survival. Recent investigations suggests that the profile of produced cytokines by immune response may influence the morbidity in patients with SCA. Haptoglobin (Hp) is a plasma glycoprotein whose primary function is to bind to free hemoglobin, preventing excretion of iron by the kidneys and protecting blood vessels from its oxidative effects. Three main genotypes (Hp1-1, Hp2-1, Hp2-2) correspond to distinct proteins with different properties. The protein Hp2-2 has the highest molecular weight and the lowest antioxidant capacity. Furthermore, Hp has immunomodulatory properties that seem to influence the pattern of inflammatory response and cytokine secretion. The Hp 1-1 genotype was associated with higher production of IL-6 and IL-10 than the Hp 2-2 genotype. Aims: Determine whether genotypes of Hp and plasma levels of IL-1β, IL-6 and IL-8 correlate with the clinical and laboratory aspects of adult patients with SCA, followed up at HEMOPE, in the state of Pernambuco, northeastern Brazil. Methods: Peripheral blood samples of 94 stable patients were collected. The Hp genotypes were determined by allele specific PCR. Results: A significant influence Hp genotype had on IL-6 and IL-8 levels when compared to the Hp 1-1 group. Patients with Hp 1-1 had significantly higher IL-6 and IL-8 levels compared with Hp 1-2 and Hp 2-2 genotypes (P = 0.01 and P < 0.001, respectively). Conclusions: In this preliminary analysis no significant differences between groups were observed in relationship to Hp genotypes, interleukins and VOC or LU. On the other hand, we have demonstrated that circulating levels of IL-6 and IL-8 were elevated in patients with SCA who developed LU. The Table 1 summarizes the partials results.

1599
INTRAVENOUS FERRIC CARBOXYMALTOSE IN THE TREATMENT OF IRON DEFICIENCY

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We have used ferric sucrose since its introduction and we report our experience with ferric carboxymaltose. The need for repeated infusions placed major strain on nursing time, Day Unit capacity and caused significant patient inconvenience. Ferric carboxymaltose offers increased dosing intervals, though at a higher drug cost. Aims To establish the efficacy of the use of intravenous ferric carboxymaltose in the treatment of iron Deficiency anaemia. Methods Over the last two years; we have treated 51 patients with a fixed dose one gram IV ferric carboxymaltose (FCM) over 15 minutes infusion. Six patients (12%) with minimal response were excluded and including patients with beta thalassaemia, pernicious anaemia and anaemia of chronic disease. Results. Table.

| No. patients | 45 (36F;9M) |
| Treatment episodes | 55 |
| Mean pre treatment Hb (g/l) | 9.9 |
| Mean post treatment Hb (g/l) | 11.2 |
| Mean pre treatment serum ferritin ng/ml | 29 |
| Mean post treatment serum ferritin ng/ml | 168.8 |
| Mean pre treatment Tsf % | 8.4 |
| Mean post treatment Tsf % | 16.9 |

Conclusions. Whilst ferric carboxymaltose carries higher prescribing costs compared with iron sucrose; it significantly reduces hospital visits, is very well tolerated and patients prefer the convenience that this drug offers. Advantages for the hospital include reduced nursing time, fewer hospital appointments and admissions, lower patient transport costs and quicker pharmacy processing, these benefits significantly offset higher drug costs. Treatments with ferric carboxymaltose were well tolerated and no adverse effects were reported and no treatment was discontinued. Conclusion: The safety and the short duration of administration of FCM may favour its delivery in the community, further improving ease of access and convenience for patients. Our study demonstrates that the use of Ferric Carboxymaltose should be targeted to the treatment of appropriate clinical conditions in order to achieve maximum patient benefit and acceptable costs.
78 patients were treated with a single dose of ferric carboxymaltose, with a weekly dose of 200 mg administered during 60 minutes and diseases and upper gastrointestinal bleeding) and 67 with gynecologic aims of this work is to compare the efficacy of therapeutic with ferric carboxymaltose was 811.86±400.47 mg. The average values of laboratory adverse reactions provides a major convenience both to patients and increasing the levels of serum hemoglobin, iron and ferritin but the effects to those obtained with iron [III] hydroxide-sucrose complex in administration of ferric carboximaltose has demonstrated to produce similar effects in the increase of serum hemoglobin, iron and ferritin to those achieved with several treatment sessions with iron [III] hydroxide-sucrose complex. However, the difference between the number of treatment sessions with one or other therapeutic formulation is significant (1.35±0.61 with ferric carboxymaltose and 3.87±1.68 with iron [III] hydroxide-sucrose complex, p<0.001). Summary/conclusions: The administration of ferric carboximaltose has demonstrated to produce similar effects to those obtained with iron [III] hydroxide-sucrose complex in increasing the levels of serum hemoglobin, iron and ferritin. But the reduced administration time of the ferric carboxymaltose, associated to the high iron uptake accomplished with a single dose and absence of adverse reactions provides a major convenience both to patients and health practitioners.

The population studied consisted in 150 patients, 83 and mean oxygen during desaturation, although these correlations did not reach statistical significance. A statistical significance was demonstrated between AHI and tonsils' size (r=0.585, p=0.026). VWF:RCo was significantly lower in patients with mild OSASH (AHI<5) compared to patients with moderate or severe OSASH (AHI≥5) (98.68 ± 17.42 vs 128.92 ± 15.01, p= 0.006). VWF:RCo activity was inversely correlated to mean oxygen during desaturation (r=-0.405, p= -0.049) and to minimum value of oxygen during sleep (r= -0.499, p= 0.014) and significant-ly correlated to AHI (r= 0.499, p=0.041). With regards to immunity parameters studied, a negative statistical trend between the expression of CD11a adhesion molecule and basal oxygen during sleep was found (r= -0.457, p=0.065). Conclusions: The study revealed a high rate of OSASH in children and adolescents with sickle/beta-thalassemia compared to other reports on sickle cell disease patients. Approximately 4% of sickle cell patients develop sickle chronic lung disease leading to end stage respiratory failure, characyterised by hypoxemia, restrictive lung disease and cor pulmonale. Such episodes start in childhood. Aims: In this study we evaluated pulmonary functions together with clinical parameters in children with sickle cell disease. Materials and Methods: 24 children with sickle cell anemia and 9 children as control group where include to the study. Complete blood count, hemoglobin electrophoresis and biochemical values were evaluated for both groups. The carbonmonoxide diffusion test perfoming for both groups. At the same day spirometric respiratory function evaluation and exercise test performed to all groups at department of sports physiology. Results: HbF, SCFT, ferritin, total bilirubine, direkt bilirubine and iron values were high at patient group (p<0.05). Hemoglobin and hematocrit values were low at patient group according to control group (p<0.05). Number of patient’s who had one-there vasoocclusive crisis were 14 (58.3%), who had 3 or more vasoocclusive crisis were 7 (29.2%) and who had no vasoocclusive crisis were 3 (12.5%). Acute chest syndrome was seen in 3 patients (20.8%). Impaired isolate carbonmonoxide diffusion test was established at the 62.5% of the patient’s. At patient group, spirometric FEV1 and MEF25 measurement were lower (p<0.05). At exercise test, oxygen uptake/heart rate were lower for patient group (p<0.05). Conclusions: Our results confirm that lung disease in sickle cell disease begins in childhood. Pulmonary function test is a major determinant of the patient’s survival. Acute chest syndrome is a catastrophic complication of sickle cell disease (SCD) and a leading cause of death in both children and adults. The risk for stroke can be inferred from abnormally high cerebral velocities assessed by transcranial Doppler (TCD) and can be reduced by cerebral levodopa administration. The annual number of painful crises and the degree of OSAS expressed as AHI, as well as with the basal oxygen during sleep and mean oxygen during desaturation, although these correlations did not reach statistical significance. A statistical significance was demonstrated between AHI and tonsils’ size (r=0.585, p=0.026). VWF:RCo was significantly lower in patients with mild OSASH (AHI<5) compared to patients with moderate or severe OSASH (AHI≥5) (98.68 ± 17.42 vs 128.92 ± 15.01, p= 0.006). VWF:RCo activity was inversely correlated to mean oxygen during desaturation (r=-0.405, p= -0.049) and to minimum value of oxygen during sleep (r= -0.499, p= 0.014) and significant-ly correlated to AHI (r= 0.499, p=0.041). With regards to immunity parameters studied, a negative statistical trend between the expression of CD11a adhesion molecule and basal oxygen during sleep was found (r= -0.457, p=0.065). Conclusions: The study revealed a high rate of OSASH in children and adolescents with sickle/beta-thalassemia compared to other reports on sickle cell disease patients. Approximately 4% of sickle cell patients develop sickle chronic lung disease leading to end stage respiratory failure, characterized by hypoxemia, restrictive lung disease and cor pulmonale. Such episodes start in childhood. Aims: In this study we evaluated pulmonary functions together with clinical parameters in children with sickle cell disease. Materials and Methods: 24 children with sickle cell anemia and 9 children as control group were included in the study. Complete blood count, hemoglobin electrophoresis and biochemical values were evaluated for both groups. The carbonmonoxide diffusion test performed for both groups. At the same day spirometric respiratory function evaluation and exercise test performed to all groups at department of sports physiology. Results: HbF, SCFT, ferritin, total bilirubine, direct bilirubine and iron values were high at patient group (p<0.05). Hemoglobin and hematocrit values were low at patient group according to control group (p<0.05). Number of patient’s who had one-three vasoocclusive crisis were 14 (58.3%), who had 3 or more vasoocclusive crisis were 7 (29.2%) and who had no vasoocclusive crisis were 3 (12.5%). Acute chest syndrome was seen in 3 patients (20.8%). Impaired isolate carbonmonoxide diffusion test was established at the 62.5% of the patient’s. 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Aims: In this study we evaluated pulmonary functions together with clinical parameters in children with sickle cell disease. Materials and Methods: 24 children with sickle cell anemia and 9 children as control group where included in the study. Complete blood count, hemoglobin electrophoresis and biochemical values were evaluated for both groups. The carbonmonoxide diffusion test performed for both groups. At the same day spirometric respiratory function evaluation and exercise test performed to all groups at department of sports physiology. Results: HbF, SCFT, ferritin, total bilirubine, direct bilirubine and iron values were high at patient group (p<0.05). Hemoglobin and hematocrit values were low at patient group according to control group (p<0.05). Number of patient’s who had one-three vasoocclusive crisis were 14 (58.3%), who had 3 or more vasoocclusive crisis were 7 (29.2%) and who had no vasoocclusive crisis were 3 (12.5%). Acute chest syndrome was seen in 3 patients (20.8%). Impaired isolate carbonmonoxide diffusion test was established at the 62.5% of the patient’s. At patient group, spirometric FEV1 and MEF25 measurement were lower (p<0.05). At exercise test, oxygen uptake/heart rate were lower for patient group (p<0.05). Conclusions: Our results confirm that lung disease in sickle cell disease begins in childhood. Pulmonary function test is a major determinant of survival. Acute chest syndrome is a catastrophic complication of sickle cell disease (SCD) and a leading cause of death in both children and adults. The risk for stroke can be inferred from abnormally high cerebral velocities assessed by transcranial Doppler (TCD) and can be reduced by cerebral levodopa administration. The annual number of painful crises and the degree of OSAS expressed as AHI, as well as with the basal oxygen during sleep and mean oxygen during desaturation, although these correlations did
has previously been associated with stroke in children with SCD. Aims: We aimed to clarify which genetic risk factors are associated with stroke in SCA in a Northeastern Brazilian population followed at Hematology and Hemotherapy Center of Pernambuco, Recife, Brazil. Methods: We have determined the α-globin genotype, the βS haplotype, and the presence of eNOS promoter -786 T[ARROWRIGHT]G, a single nucleotide polymorphism (SNP), TNF promoter -308 G[ARROWRIGHT]A, GATA factor (GATA1), and MTHFR C677T point mutations and G6PD202A mutation of patients with proven SCA as confirmed by hemoglobin HPLC pattern. All molecular analyses were determined by PCR-RFLP, except for the α-globin genotypes, which were determined by gap-PCR. Statistical analyses used Fisher’s exact test. Results: A total of 168 patients with SCA were included in our case-control study. Of these, 53 patients presented clinical signs and symptoms of stroke, defining the case group of this study. The control group consisted of 115 patients presenting normal TCD with absence of clinical signs and symptoms of stroke. The α-gene deletion (α-α7Kb) showed significant difference (p=0.0008) between case and control groups (3 patients, 5.7% vs. 32, 27.8%, respectively), corroborating the previously reported protective effect of α-gene deletion in stroke. The CAR/βS haplotype group had statistically higher frequency in the case group compared with the control group (p=0.0174). CAR/βS individuals appear to be at higher risk for stroke than other patients. We found no difference in the other polymorphic genotypes comparing patients with and without stroke in our SCA population. Conclusions: From all genetic markers in our study, only α-globin genotype and βS haplotype showed reproducible influence as genetic modulators for stroke prevalence in our SCA population. This demonstrates that genetic heterogeneity among different ethnicities account for failure to prove reproducibility of previous gene association studies, and warrants further studies in worldwide collaboration to determine the actual relevance of findings involving genetic polymorphisms and their influence on the prevalence and predictability of clinical complications in SCA.

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1605
THE NEW ORAL COMBINED CHELATION REGIMEN MAY IMPROVE SHORT STATURE AND PUBERTAL MATURATION IN JUVENILE thalassemia major patients
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Iron overload has a critical impact on multitransfused thalassaemia major patients (TMs) inducing significant morbidities. Chelation with deferoxamine mesylate (DF) and deferasirox (DFX) offer the promise of easier administration, better compliance without any toxic adverse effect in lower ferritin levels. Aims. The substitution of a chelation regimen to daily DF for 3 weeks in TM patients with a normal pubertal growth spurt in time. Additional objectives were to evaluate the efficacy on total body iron load and the safety of the oral combined chelation. Patients and Methods. 3 juveniles TM, 1 male 2 females, mean age 11.5±2.5, were included in the study for a two years period. Hospital’s Ethics Committee approval and appropriate written informed consent from guardians were obtained prior to participation. The protocol involved the combination of two oral chelators DF: 100 mg/kg/day and DFX: 20 mg/kg/day. Primary endpoint measures were investigated by growth-charts for age percentiles and clinical staging of puberty according Tanner criteria. Regarding efficacy, yearly mean serum ferritin levels (CMIA), quantification of heart and liver iron by eGFR calculated by Schwartz formula. These results indicate that oral combined chelation (DFP and DFX), seemed to be beneficial in juveniles TM for attaining normal stature and sexual maturity. Additionally it improved cardiac function. It is also well tolerated, more acceptable for life-long chelation and influences significantly patients’ quality of life.

1606
INVASIVE MOLD INFECTIONS (IMI) IN PATIENTS WITH ACUTE LEUKEMIA RECEIVING CHEMOTHERAPY
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Background. Acute leukemia patients on chemotherapy receive intravenous prophylaxis and are treated according to a febrile neutropenia guideline developed based on the hospital’s flora and antibiotic resistance. Aims. To determine risk factors for and clinical features of invasive mold infections(IMI) in patients with acute leukemia receiving chemotherapy. Methods. Patients diagnosed with acute leukemia between 1st January 2004 and 31st March 2007 and treated with chemotherapy were included in the study, approved by the Institution Review Board. A retrospective review of their casenotes was made to establish patients’ clinical profile, disease characteristics and IMI management. These cases were then matched to controls obtained from the Leukemia Registry. Results. Nineteen cases of invasive mold infections (IMI) were found in the period, giving an incidence of 11.2%, using only patients undergoing curative chemotherapy as denominator. The incidence in patients receiving the first cycle of chemotherapy was 8.9%; in patients receiving subsequent cycles of chemotherapy was 4%. Thirteen cases were proven by EORTC/MSG criteria, 8 of them by histology with positive cultures. There were 3 proven cases of fusariosis, and 1 each of aspergillosis and nodulisporiosis. Cases were more likely than controls to have had an absolute neutrophilia for at least 14 days in the chemotherapy episode in which the IMI was diagnosed (p=0.045), to have been bacteremic prior to the diagnosis of IMI (p=0.045), and to have had fever not responding to a carbapenem (p=0.016). They also had a longer length of stay (51.8 vs 27.2 days, p=0.01). The use of antifungal prophylaxis was not less common in cases than controls. Common clinical features noted in cases included fever not responding to a carbapenem (84.7%), cough (42.1%), a rise in alkaline phosphatase(ALP) (36.9%), breathlessness (26.3%), and hemoptysis (21.1%). A pleural effusion was noted on CXR or CT chest in 52.6% of cases, and a characteristic chest radiograph in 47.4% of cases. Sixty-eight percent of cases received amphotericin (conventional or lipid preparation) as part of a febrile neutropenia protocol, though only 15.8% received this agent when an IMI was considered. Clinical improvement was attributed to an increase in ANC in 26.3% of cases, and to the introduction of voriconazole in 10.5%. The next cycle of chemotherapy was delayed for 2-4 weeks in 15.8% of cases, and for more than 4 weeks in 31.6% of cases. Three patients (15.8%) died within three months of IMI diagnosis, 47.4% survived more than 1 year. Conclusions. Absolute neutropenia more than 14 days is a risk factor for IMI in patients with acute leukemias. Fever not responding to carbapenems, and a rise in ALP may be considered red flags for clinicians to consider the possibility of an IMI. The anti-fungal prophylaxis used during the period under study (itraconazole) did not reduce the likelihood of an IMI and a change should be considered.
 **I. Ragab, G Mokhtar, S El Sayed**

**ENCE ADMISSION AND SENSITIVITY PATTERNS; A SINGLE CENTER EXPERIENCE**

Cerrahpasa Medical Faculty, Medical Biology Department, Istanbul, Turkey

50 children with cancer and receiving chemotherapy was performed; 22 patients attending the clinic. A case-control study of fungal colonization in Ain Shams University Pediatric Oncology and compare them to outpatients. Frequency of fungal colonization; fungal species characterization and antifungal sensitivity in pediatric oncology inpatients admitted at the Hospital Príncipe de Asturias, Alcalá de Henares, Spain

**1608 FUNGAL COLONIZATION IN CHILDREN WITH CANCER: HOSPITAL ADMISSION AND SENSITIVITY PATTERNS; A SINGLE CENTER EXPERIENCE**

I. Ragab, G. Mokhtar, S. El Sayed

Ain Shams University, Cairo, Egypt

Background: Fungal infections represent a growing problem in children with cancer and fungal colonization is recognized as an important risk factor for these infections. Aim: The aim of the study was to detect the frequency of fungal colonization; fungal species characterization and antifungal sensitivity in pediatric oncology inpatients admitted at the Ain Shams University Pediatric Oncology and compare them to outpatients attending the clinic. A case-control study of fungal colonization in 50 children with cancer and receiving chemotherapy was performed; 22 patients admitted for febrile neutropenia compared to 28 outpatients as a control group. Each patient was evaluated for the occurrence of fungal colonization (defined as at least one positive surveillance culture). Samples were collected by swabbing both buccal mucosa, axillae in all patients, in addition to blood and stool studies during peak of fever, and perianal sample in the presence of inflammation or rash. All samples were cultured on Sabouraud’s dextrose agar, thereafter, susceptibility of patients, in addition to blood and stool studies during peak of fever, and colonization (defined as at least one positive surveillance culture). Swabbing was performed by hybridization probe assay specific for the TLR2 variant. Informed consent was obtained. Results: The mean age of the patients was 5.9 years old (±3.7 years), 52% were male. According to BFM-95 treatment protocol risk groups; 35.3% of the patients were in standard, 58.3% in medium and 8.3% in high risk group. During initial treatment (BFM-95) 8 (8.3%) IFIs were observed. According to the revised definitions of IFIs; 4 were proven, 2 probable and 2 possible. Leukemia relapsed in 15 patients, other 4 patients (26.6%) had IFI during relapsed ALL treatment protocol (BFM Rez-ALL). TLR2 753Gln mutant allele was found as heterozygous in 1 patient with ALL, this patient had no febrile neutropenia attack. IFI or any serious infection during the treatment. TLR2 Arg753Gln polymorphism was not observed in controls. Conclusions: Limited data is available on the incidence of TLR2 mutation so no epidemiological conclusions can be drawn. However, interestingly the only patients who was heterozygous had no febrile neutropenia attack, IFI or any serious infection during the treatment. The known TLR2 polymorphism identified so far may not cause a crucial role in the pathogenesis of IFIs in children treated for ALL.

1609

**INVASIVE FUNGAL INFECTIONS IN LYMPHOPROLIFERATIVE DISORDERS**


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Background: Few epidemiological data regarding invasive fungal infection (IFI) in chronic lymphoid malignancies are available in literature. Aims: The aim of our study is to describe epidemiology, clinical manifestations and outcome of IFI in these patients. Methods: We reviewed the records of patients (pts) affected by lymphoproliferative disorders, admitted to our department between 2005 and 2010 and treated for probable or proven IFI. Results: We have prospectively registered 34 probable and proven IFI. In our population, the incidence of IFI was 2.7% (moulds 2%, yeasts 0.4%, mixed infections 0.3%). Twenty-two patients were affected by lymphoma, 8 by chronic lymphocytic leukemia, 3 by Waldenström macroglobulinemia and 1 by hairy cell leukemia. The median age was 57 years (7-71). Twenty-six patients (76%) had progressive or relapsed hematological disease, and 76% was treated with multiple lines of chemotherapy. According to the criteria of EORTC/MSG, risk factors were: deep and prolonged neutropenia (10 pts), immunosuppressive therapy after solid organ transplant (2), previous allogeneic HCT (2), high dose steroid therapy (5), monoclonal antibody therapy (5) and rituximab (2). Every patient received at least one antifungal agent. Among these, 6 patients died due to a progressive hematological disease (2) or multiple of these risk factors (8) during the previous 90 days. Six patients developed a yeast infection; 4 cases infection was documented by blood cultures (2. C. albicans, 1 C. glabrata and 1 B. bassiana), 6 patients died due to a progressive hematological disease. In 6 patients, infection (2 by yeasts, 4 by moulds) was a concurrent cause of death. In 1 case IFI with mixed etiology (Candia spp/Aspergillus spp) was the only cause of death as documented at autopsy. Fungal attributable mortality was 20% (7/35). The only significant risk factor for mortality at univariate and multivariate analysis was the persistence of neutropenia.

1610

**HAS NOVEL INFLUENZA A/H1N1 2009 ASSOCIATED PNEUMONIA POTENTIAL TO BE MORE PATHOGENIC IN ADULT PATIENTS WITH HEMATOLOGICAL MALIGNANCIES? CASE REPORTS AND REVIEW OF THE LITERATURE**

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Background and aims: During the recent winter, the northern hemisphere, especially Europe, experienced a second wave of novel influenza A/H1N1 2009 virus. A devastating complication of this viral infection is pneumonia. In hematological malignancies (HM), however, no studied has comprehensively evaluated the novel A/H1N1 associated pneumonia outcome during this second wave. To address this issue, we analyzed the clinical cases described in the English literature. HM from our department with novel A/H1N1 2009 virus pneumonia were also included in the analysis. Methods: MEDLINE was searched using the following key words: 2009 A/H1N1 influenza, HM
and HSCT. Results: The search identified 25 cases of A/H1N1 associ-ated pneumonia (Table 1). All these reports were dated during the initial wave (April 2009-March 2010). Mean age was 43 years (range 15-72). The most common underlying HM was AML (32%) followed by NHL (20%). Sixteen (64%) had previously undergone allo-HSCT. The medi-an absolute lymphocyte and neutrophil counts were 625 cells/µl (20-8800) and 300 cells/µl (10-6020), respectively. All patients were treated with oseltamivir 150 mg daily for 5-10 days. The median time from illness onset until the start of oseltamivir was 3.5 days (1-22). Critically ill patients were also receiving combinations of antibiotics and anti-fungal treatment. Eight (32%) patients required mechanical ventilation. Mortality rate attributable to novel A/H1N1 pneumonia was 19%. On the other hand, during the second wave (December 2010- March 2011), no cases of novel A/H1N1 associated pneumonia have been reported in HM. Herein, we present the first two known cases (both died due to influenza). Patient 1, a 55-year-old woman presented with typical flu symptoms and bilateral pulmonary infiltrates. On admission showed leukocytes 5.6 x 10^9/L with 80% blasts. A diagnosis of AML FAB M2 was made. Novel A/H1N1 virus was detected by RT-PCR in both nasopha-ryngeal swab (NPS) and BAL. Osel tamivir 75mg/12h started on day 3; however she died in ICU 10 days later due to respiratory failure despite broad spectrum antibiotics. Patient 2, a 52-year-old woman diag-nosed with Multiple Myeloma in June-2010, achieved complete remis-sion after a bortezomib-based combination. However, on February 2011, she experienced a meningeal relapse. While undergoing chemo- and radiotherapy she developed flu symptoms and alveolar infiltrate in right upper lobe. NPS specimen showed novel A/H1N1 virus by RT-PCR. Two days after the onset of symptoms oseltamivir 75mg/12h and antibioti cs were started. For the next 6 days she developed worsening respira-tory distress and died 5 days later. There was no correlation between severe lymphocytopenia and mortality risk.

Conclusions: During the second season of the novel A/H1N1 pandem-ic, it appears that HM with novel A/H1N1 associated pneumonia might present with a high mortality (100% vs 19% during the initial wave) despite early antiviral treatment. Additional studies are needed to con-firm these data. Thus, prevention strategies (infection control and vac-cination) for patients, family members and caregivers are clearly indicat-ed.

1611
A FULMINANT COURSE OF INFLUENZA A (H1N1) VIRAL PNEUMONIA IN A PATIENT WITH MULTIPLE MYELOMA AFTER TREATMENT WITH BORTEZOMIB
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Background. In 2010, 63 deaths and 2193 hospital admissions were reported in the Netherlands due to the current pandemic of Influenza A (H1N1). Main victims were children aged 0-5 years old, pregnant women and immunocompromised patients. Interestingly, in a recent study of 92 hospitalized patients with flu-like symptoms, 6/13 H1N1 PCR-positive patients received treatment for multiple myeloma [Garland, BBMT 2010]. Oseltamivir for influenza is not indicated for oncology patients. Herein, we present a case of a patient with multiple myeloma treated with oseltamivir. Case report. A 52-year-old man with multiple myeloma completed 3 monthly induction courses consisting of bortezomib (d1,4,8,11), doxorubicin (d1-4) and dexamethason (d1-2). His renal function had improved and serum IgG lambda had decreased from 25 to 11 g/L. Three weeks later he was admitted with fever, coarseness and dyspnea. He was treated with broadspectrum antibiotics. All cultures remained negative. Over the next week he developed diarrhea and respiratory insufficiency. The diagnos-is of H1N1 was made and antiviral medication was started. Before bortezomib, peripheral blood levels of T-cells and NK-cells were normal (CD8+ 0.8x10^9/L, CD4+ 0.9x10^9/L and NK 0.06x10^9/L). Immunopheno-typing at diagnosis of H1N1 showed slightly elevated CD8+-T-cells (1.0x10^9/L), but diminished CD4+-T-cells (0.37x10^9/L) and NK-cells (0.03x10^9/L). Broncho-alveolar lavage showed a particularly high copy number of viral RNA (CT value 21.9).

He was supported with mechanical ventilation, inotropic drugs and haemodialysis, but he finally died of lung injury, multi-organ failure and secondary sepsis. On autopsy, the lung parenchyma showed diffuse alveolar damage with hyaline membranes and edema. Immunostaining demonstrated influenza viral antigen in alveolar epithelial cells and alveolar macrophages in lesional lung tissue. H1N1v virus was detected throughout the respiratory tract as well as in brain and jejunum. Virus histochemistry demonstrated that H1N1v virus was also able to repli-cate in human alveolar epithelial cells. Conclusions. This fatal case of H1N1 demonstrates a rare finding: viral infection and replication of H1N1 in the alveolar epithelium. Furthermore, bortezomib treatment may lead to lung injury by uncontrolled CD8+-T-cell activity. Therefore, multiple myeloma patients may be at increased risk of a more ful-minant course of H1N1 viral pneumonia.

1612
DISSEMINATTED CRYPTOCOCOSIS RESSEMBLING DISEASE PROGRES-SION IN A PATIENT WITH A HLTV-1 ASSOCIATED ADULT-T-CELL LEUKEMIA-LYMPHOMA IN THE COURSE OF CHEMOTHERAPY ADMIN-ISTRATION
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Background. HTLV-1 retroviral infection is endemic in African coun-
The incidence of HSV, formed in leukocytes by gene-specific PCR at a weekly basis, up to day 10 after conditioning therapy was 1/2. Combination treatment with liposomal Amphotericin B and Flucytosine was programmed for the opportunistic infection with pathogen identification. Conclusions. Under the presence of hypocalcaemia and pulmonary nodules in patients with HTLV-1 infection, under chemotherapy and antiretroviral treatment are mandatory exclude opportunist infections.

**1613 EARLY REACTIVATION OF HERPESVIRUSES IS ASSOCIATED WITH COMPLICATIONS OF HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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Background. Cytostatic therapy preceding HSCT causes common infectious complications, including reactivation of viral and fungal pathogens. Aims. The aim of our study was to study possible associations between herpesvirus reactivation and common complications following HSCT. Patients and methods. A group of 143 patients at the age of 2 to 61 years old (a median of 18.5 years) with various oncohematological disorders underwent allogeneic HSCT. HSCT, 64% of cases; myeloablative conditioning was chosen in 58%. Common complications, i.e., acute GVHD, mucositis, cystitis, pneumonias, neurological disorders, severe general infections were registered post-HSCT. The study included a sub-group of 54 children and young patients up to 21 years old with ALL and AML who were subject to HSCT with myeloablative (n=31) or non-myeloablative conditioning regimens (n=23). DNA testing for cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus (EBV), Candida albicans, and Toxoplasma gondii was performed in leukocytes by gene-specific PCR at a weekly basis, up to day +100. Results. In general group of the patients, the incidence of HSV, CMV and EBV after HSCT was respectively 51%, 57% and 45%. Meanwhile, frequency of viral reactivation proved to be age-dependent, i.e., HSV and CMV positivity rates were lower in younger children (1 to 4 years old), followed by increased viral reactivation rates at 10-20 years. Strong correlation was found between CMV and HSV, as well as CMV and EBV reactivation, thus suggesting mixed infectious states. We have not revealed any differences in viral reactivation among patients who underwent conditioning therapy of different intensity. However, a higher percentage of infectious complications was found among young HSV-positive patients vs HSV-negative cases (resp., 88% and 59%, p=0.057). In younger patients (<21 years), a correlation was found between HSV persistence and neurological symptoms (P<0.002). Skin mucositis severity and duration was connected with HSV and CMV reactivation (P=0.02, or 0.008). Risk of acute intestinal GVHD and hemorrhagic cystitis correlated with EBV reactivation (P=0.01). Polymavirus DNA (BK, JC) was detected in urine sediments, and positive findings, generally, reflected clinical signs of cystitis. Conclusions. Post-HSCT reactivation of HSV in younger patients is significantly associated with higher incidence of early complications, including bacterial infections, skin mucositis and neurological complications.

**1614 GRANULOCYTE TRANSFUSIONS AS ADJUNCTIVE TREATMENT OF INFECTIONS IN NEUTROPENIC PATIENTS**

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Background: The degree and duration of neutropenia have been recognized as crucial prognostic factors in hematological patients with invasive infections. Since the introduction of granulocyte colony stimulating factor (G-CSF), there has been a renewal of interest in granulocyte transfusions (GTX) in this setting. However, there is a large variability in the transfusion strategy and uncertainty about the beneficial effects of GTX as adjunctive measure to antimicrobial therapy. Aims: We designed a retrospective analysis to evaluate feasibility, efficacy and safety of GTX as adjunctive treatment for neutropenic fever unresponsive to antimicrobial therapy. Methods: We conducted a retrospective analysis about adult patients with hematological malignancies (HM) and fever during neutropenia (ANC<500x10⁹/L) and anticipated duration >7days who received GTX after no clinical response to antimicrobial therapy. Volunteer donors received G-CSF 12h before the first of two consecutive collection procedures (5μg/kg). All of them had signed an informed consent for G-CSF administration and leukapheresis. Results: During a 7-year period (2004-2010) 46 patients of GTX were administered to patients with neutropenic fever, after no clinical response to antimicrobial therapy. Patients were suffering from acute leukemia (30 myeloid and 5 lymphoid), lymphoma (9), multiple myeloma (2). Overall, 209 GTX were administered, with a median of 4 GTX per episode of infection (range 1-20). Infections causing fever were identified in 41 episodes: 17 bacterial sepsis, 25 invasive fungal diseases (IFDs) and 1 mixed bacterial/fungal sepsis. Remaining 5 cases were classified as fever of unknown origin (FUO). IFDs included 16 cases of pulmonary aspergillosis (proven/probable), 5 candidiasis, 1 invasive zygomycosis, 1 invasive fusariosis and 1 infection due to Blastoschizomices capitatus. Donors’ mean white blood cell (WBC) count at first collection was 27x10⁹/L (range 13-45); at second procedure the WBC count was lower (15x10⁹/L, range 8-38), as expected. The mean yield was 25.6 x 10⁹ PMN (range 3.5-75.8) at first procedure and 11.1 x 10⁹ PMN at the second one (range 0.6-42.4). Mean transfused dose was 3.7 x 10⁹/kg at first day (range 0.6-9.6) and 1.4 x 10⁹/kg at second day (range 0.1-4.7). The combination of antimicrobial therapy with GTX led to a favourable clinical response in 53 of 46 valuable patients (72%); the acute infection-attributable mortality rate at 30th day after the last GTX was 29% for sepsis, 22% for IFD and 40% for FUO. Conclusions: at preliminary analysis GTX may be safe and efficacious in HM with severe infection to bridge the period of deep neutropenia, when antimicrobial therapy has failed. Controlled studies are needed to confirm this datum, and to define the proper role of this procedure and the optimal schedule for HM.

**1615 PIPERACILLIN/TAZOBACTAM + AMIKACIN VS CEFEPIME + AMIKACIN AS EMPIRRICAL ANTIBIOTIC THERAPY IN NEUTROPENIC PATIENTS. A SINGLE CENTRE RETROSPECTIVE ANALYSIS**

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Background: Hematologic patients (pts) are at high risk for infections because of the disease and the chemotherapy that could induce a pro-inflammatory and deep neutropenia. Aims: The aim is to evaluate the efficacy of two different empirical antibiotic regimens. Methods: Between 2001 and 2006, 316 consecutive pts were included. At the beginning of chemotherapy they were randomly assigned to receive piperacil- line/taizobactam (162 pts, Group 1) versus cefepime (154 pts, Group 2) + amikacin. All received levofloxacin as prophylaxis. In case of fever (pts with high clinical suspicion were diagnosed and each patient received the assigned regimen. The 2 groups were similar for gender, age and disease’s phase. The diagnoses were for each group respectively: leukemia 74 and 63; lymphoma 27 and 37, myeloma 56 and 41; the remaining diseases (aplastic anemia, multiple sclerosis, solid tumors) were similar in the 2 groups. 140 pts received a SCT (108 autologous and 32 allogeneic); the transplants were homogeneously distributed in the 2 groups. A CVC was present in 113 pts (70%) of group 1 and in 110 pts (72%) of group 2. The days with PMNs <0.1 x 10⁹/L were 10 (range 1-
A MILIARY TUBERCULOSIS CASE ACCOMPANIED BY HEMOPHAGOCYTOSIS AND PANCYTOPENIA

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Background: Miliary tuberculosis occurs through uncontrollable spread of mycobacterium tuberculosis via lympho-hematogenous route. The symptoms of tuberculosis are quite variable; primary infection tends to be acute whereas late miliary tuberculosis is frequently subacute or chronic. The disease could go unnoticed because of the non-specific symptoms such that in USA, it was reported that %20 of cases of miliary tuberculosis had a post-mortem diagnosis (2). Methods: A 25-year-old male patient sought medical help for fever, fatigue, weight loss and night sweats for three months; his fever was uncontrolled despite antibiotic and antifungal therapy. Hepatosplenomegaly and multiple cervical lymphadenopathies were detected in physical examination. His hemogram was Hg: 6.9 g/dl, Hct: 20.6%, Plt: 125,000/μL. Besides, liver function tests followed a high and fluctuating course; AST: 110 U/L, ALT: 129 U/L.

Results: We decided to perform splenectomy, liver wedge biopsy and bronchoscopy and brush biopsy were normal. Transthoracic Echocardiogram was normal. In follow-up, pancytopenia developed; Hemoglobin: 5.8 g/dl, WBC: 1700/μL, Neutrophile: 1100/μL, PLT: 59000/μL. Besides, liver function tests followed a high and fluctuating course; AST: 110 U/L, ALT: 129 U/L.

Conclusions: The response in the 2 groups is similar in terms of days of fever, need for further antibiotic courses, need for antifungal therapy and resolution of fever even if further and larger studies are necessary to assess if one regimen is superior.

IMPORTANCE OF PRE-MEDICATION IN DECREASING THE INCIDENCE OF INFUSION-RELATED REACTION BEFORE USE OF AMPHOTERICIN B COLLOIDAL DISPERSION IN PATIENTS WITH ACUTE LEUKEMIA

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Background: Fungal infections cause significant morbidity and mortality in patients with acute leukemia (AL). Conventional amphotericin B deoxycholate (AmB) has been successfully used for treatment of these infections. Nevertheless, the side effects and particularly nephrotoxicity of AmB limits its use. Amphotericin B colloidal dispersion (ABCD) has decreased the rate of nephrotoxicity, but it seems that the frequency of infusion-related reaction (IRR) is still high in comparison to other forms of AmB, despite the pre-medication received before its use. The most commonly reported IRRs were chills, rigor, fever, nausea and vomiting. Aim: The aim of this study was to determine the type and the incidence of IRR in patients with AL who were receiving ABCD according to the type of pre-medication used as well as ABCD’s doses. Methods: In this study 32 patients with AL, receiving ABCD, were included. Indications for ABCD use were empirical in prolonged febrile neutropenia or therapeutic in documented fungal infection. Patients were divided into 2 groups according to the 2 types of pre-medication applied. Each group involved 16 patients. One group received type 1 pre-medication: methylprednisolone 300 mg + metamilzol 2.5 mg intravenous (i.v.) 30 minutes before infusion of ABCD. The other group received type 2 pre-medication: methylprednisolone 40 mg i.v. + loratidine 10 mg per os (p.o.) + paracetamol 1000 mg p.o. 30 minutes before infusion of ABCD. Applied doses of ABCD were 2.5-5 mg/kg. Duration of IRR in days (started after first infusion of ABCD) were recorded. The median duration of ABCD’s treatment was 9 days. The risk factors were identified using the univariate and multivariate...
al.): Immature Neutrophils are bigger than segmented neutrophils (Woessner et al.). They look for similar aspects of neutrophils, because it is well known that immature neutrophils (AUC 0.634) had a lower performance than Mean Neutrophil Volume (CPD) (AUC: 0.922) even if they were found a discriminant function for sepsis (DFS=1.12 - 0.004* NE# - 0.065* HGB + 0.013* MCV - 0.019* MNS + 0.025SD - S-Ly) with 100% sensitivity, 100% specificity with AUC 0.889, at a cut-off >0.3242 (Table 2). Immature neutrophils (AUC 0.634) had a lower performance than Mean Neutrophil Volume (CPD) (AUC 0.922) even if they look for similar aspects of neutrophils, because it is well known that immature neutrophils are bigger than segmented neutrophils (Woessner et al.). Summary: These findings demonstrate that the automated leukocyte morphology (CFD) and the combination of CFD and other parameters may dramatically improve the early detection of neonatal sepsis. Further studies could evaluate IL-6 to see if IL-6 could further enhance the predictive value. Also, prospective protocols on a larger number of cases and/or randomized co-operative studies could be designed to prove the clinical efficacy of the present findings so as to incorporate them in daily clinical practice.

1619
A SURVEY ON CMV REACTIVATION IN 102 PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: EFFECTIVENESS OF PREEMPTIVE THERAPY

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Background: Cytomegalovirus (CMV) continues to cause major complications after allogeneic stem cell transplantation (allo-SCT). Materials and methods: In 2009 and 2010, 102 pts (median age 49; 68 males) received transplant for high risk haematological malignancies in our centre: 51 AML, 14 ALL, 2 biphenotypic AML, 6 MDS, 10 HD, 6 NHL, 3 CML, 3 MFI, 7 other diseases. Allo-SCT were performed in 96/102 from PBSC source; 10 from HLA identical sibling (Sib), 21 from unrelated donor (MUD), 65 from family haploidentical donor (Haplo) without ex-vivo T-cell depletion, 6 cord blood (CB). Pts were evaluated for CMV quantitative DNA PCR twice weekly within the first 3 months after allo-SCT. Donor/host serostatus was: +/+ (8 %), +/- (51 %), -/- (32 %), +/- (9 %). All pts received acyclovir as viral prophylaxis. Results: We observed CMV reactivation in 59/102 pts (58%), median time of onset 31.5 (3-91) days post Allo-SCT, median number of CMV PCR 1377 (93-346480) cp/mL. The donor/host (D/H) serostatus in these pts was +/+ (56%), +/- (39%), +/- (5 %); 32 were Haplo, 5 CB, 15 MUD, 7 Sib allo-SCT. Preemptive therapy was administered in 39 pts; 13 pts received oral ganciclovir (GCV) as first line preemptive therapy, 17 ganciclovir (GCV), 9 foscarnet (FCV). Median time of CMV infection was 28 days (5-407) days and we observed a median of 1 CMV reaction in the first year post transplant; these events were similar in all pts of this group. A total of 13 pts required drug cross-over; D/H serostatus was neg/pos in 9 pts, pos/pos in 4. Only 2 cases of CMV organ involvement were observed after MUD allo-SCT: in one the site of CMV disease was colon in the other lung. In these pts who experienced a CMV reactivation 37 are still alive, 22 were dead. No CMV reactivation was reported in 45/102 pts (42%). The donor/host (D/H) serostatus in these pts was: +/+ (19 %), +/- (23 %), -/- (5%); 33 were Haplo, 5 CB, 6 MUD, 3 Sib allo-SCT. In these pts who not experienced a CMV reaction 21 are still alive, 22 were dead. Conclusions: These data suggest the efficacy of CMV prevention in HCT recipients from all donor sources.

1620
INCIDENCE OF INFUSION-RELATED REACTIONS IN PREMEDICATED PATIENTS RECEIVING AMPHOCIL®: A SINGLE CENTER EXPERIENCE

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Background: Higher rate of infusion-related reactions (IRRs) occurring with Amphotericin B Cholesteryl Sulfate Complex for Injection (ABCBD; Amphotec®/Amphocyl®) vs. other forms of amphotericin B are report-
ed. It has been recently shown that premedication prior to ABCD decreases the incidence of IRRs. Aim: To determine the IRRs incidence associated with Amphocil® therapy in the context of the premedication. Patients and Methods: Patients receiving therapy with Amphocil® were eligible. They were treated as follows: 1mg/kg first day and then 3-4mg/kg (maximum 6mg/kg); i.v infusion. At least two premedication drugs were recommended (ad libitum). The patients has received the same premedication combinations during the treatment. In the case of IRRs the infusions were stopped and Acetaminophen and/or Meperidine was administered. Corticosteroids were reserved for grade III of IRRs. Data on ABCD doses and premedications have been collected during the whole treatment period. Data collected included: demographic, co-infections, history of prior antifungals, routine laboratory values, type of fungal infection, premedication type, daily dose of ABCD and IRRs occurrence/grade/type. Informations regarding the patients response to ABCD were collected. Mann-Whitney test was used to compare the rates of IRRs between various premedication regimens. Results: 39 adult patients (M-F/25:14; median age 40 yrs, range 15-65; median body weight 79 kg, range 45-118) were treated with Amphocil® from March 2007 to June 2008. The majority had acute leukemia 28/39; 29/39 received prior antifungals (conventional amphotericin B 14, azoles 8). Two patients had definite, 6 probable and 17 possible fungal infections, respectively. Lungs were the most frequent infection site (21); 14 pts were treated with Amphocil® empirically. There were 351 infections in total, range 1-25; average daily dose was 3.46 mg/kg, range 1.7-5.80; average cumulative dose was 2513 mg, range 100-10300; average therapy duration was 9 days, range 1-35. All the patients were premedicated - 19 received two, 20 three drugs. The premedications used were as follows: Acetaminophen, Loratadine, Meperidine, Dexamethasone, Methylprednisolone, Phenylbutazone. The most frequent combinations of premedications were: Acetaminophen + Loratadine in 9 and Acetaminophen + Loratadine + one corticosteroid in 11 pts. 20/39 pts. experienced IRRs while IRRs were reported following 48 infusions (15.7%). The most frequent IRRs were: chills 40/48, fever 42/48 and rigors 16/48. All IRRs were low severity. The incidence if IRRs was highest in the first five days of therapy (d1: 17, d2: 13, d3: 7, d4: 4, d5: 2, d6: 1). Loratadine was associated with a statistically significant lower rate of IRRs vs. other types of premedication (p<0.03). Reasons for Amphocil® discontinuation were as follows: recovery (17), death (10), identification of fungal organism (6), infusion intolerability (5) and infusion intolerability associated with recovery (5). No significant nephrotoxicity was registered. After 12 weeks of follow-up 27 pts were alive, all positively responding on Amphocil®. Conclusions: We demonstrated lower rates of IRRs compared to historical rates which, is in agreement with the results of ProACT. The incidence of IRRs decreased significantly from days 1-5 and disappeared after day 7. Premedication with Loratadine was associated with significantly lower IRRs rate comparing with other type of drugs.

1621
INFLUENZA A H1N1 VIRUS INFECTION IN PATIENTS UNDERGOING HSCT

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Background. Hematological patients (pts) under hematopoietic stem cell transplantations (HSCT) are at a high risk for severe viral infections. We analyzed the clinical impact of influenza A infection (H1N1) outbreak among HSCT recipients. Material and Methods. From September 2009 to January 2011 we reported 7 cases of H1N1 infections in 7 HSCT recipients. Patients’ characteristics are shown in table 1. Diagnosis of H1N1 was made by real-time PCR assay in nasopharyngeal samples. Results. Six pts were not vaccinated, one developed the first symptoms of influenza 19 days after the first dose of vaccine. The most frequent symptoms were: fever (100%), cough (86%), dyspnea (43%), rhinorrhea (28%), odynophagia (28%), headache (20%) and arthralgia (14%). All cases were confirmed by PCR in nasopharyngeal or bronchoalveolar lavage samples. Five pts had pneumonia; CT-scan showed multiple pulmonary nodules with ground glass appearance. Chest X-ray results were discordant. All patients needed oseltamivir 75 mg bid for a median of 7 days (7-19), except for one who received oseltamivir 150 mg bid for the severity of the infection. Oseltamivir was started within 24 hours from the onset of symptoms. All pts received concomitant antimicrobial therapy for a median of 17 days (7-40). No major adverse events were reported. This study highlights the anti-influenza treatment was reported. We observed a decrease of platelet counts in four pts with pancytopenia at the time of infection, 3/4 pts required transfusion support. Resolution of symptoms was achieved in a median of 14 days (8-30) in 5/7 pts. Four pts needed hospitalization for a median of 15 days (10-15); 3/4 pts needed ventilatory support (C-FAP for 1D1, C-FAP plus intubation for 1D3, high-flow O2 therapy for 1D4). All pts developed lymphopenia (<1000/mcL). Two pts died from respiratory failure: one was in progression disease, one other patient was in complete remission but profoundly immunosuppressed (high dose steroids plus Cyclosporin as GVHD therapy). Conclusions: H1N1 infections in HSCT recipients can result in a severe and fatal syndrome. Since symptoms of a H1N1 infection are unspecific, early testing for H1N1 virus in hematological pts is mandatory. CT scan is the diagnostic investigation of choice to rule out pneumonia. Clinical courses of H1N1 infections in HSCT recipients range individually. The early use of oseltamivir may help determining the good outcome of the infection, but prognosis seems to be strongly related to the disease status and to the degree of immunosuppression. In our experience lymphopenia correlates with the outcome.

1622
HUMAN HERPESVIRUS-6 (HHV-6) REA CTIVATION IN HEMATOLOGICAL PATIENTS

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Background: Human Herpesvirus-6 (HHV-6) can reactivate and sustain various clinical manifestations in immunocompromised patients (pts). Materials and methods: We retrospectively evaluated hematological pts who developed positivity to HHV-6, measured by quantitative PCR. Results: From January 2009 to January 2011 we observed HHV-6 positivity in 49 pts (median age 54; 30 males), 39 of them received allo- or autogeneic stem cell transplantation (allo-SCT), 2 autologous transplantation, chemotherapy. At the time of reactivation all pts were receiving acyclovir as viral prophylaxis except 5 (3 off antiviral therapy, 2 on ganciclovir). A concomitant CMV positivity was detected in 11/49 pts, while a severe neutropenia in 23/49 pts. Allo-SCT was performed in 38/39 pts from PBSC source; 5 from HLA identical sibling, 3 from unrelated volunteer, 29 from haploidentical donor, 2 cord blood. Among allo-SCT pts 15 had GVHD (15/15 with grade III-IV aGVHD), and 32 were profoundly immunosuppressed with variable association of 2-4 drugs. Median time from allo-SCT to HHV-6 reactivation was 41 days (7-625). In 25 pts HHV-6 was detected in plasma, with a median number of 19937 cp/mL (34.4524600); 18/25 pts had fever (9 bacterial and 1 fungal infection), 8/25 skin rash, 4/25 worsening of liver function, 5/25 cytopenia. In 9 cases HHV-6 was present in bone marrow samples and 5 of them had concomitant HHV-6 plasma positivity; the median viral load was 25125 cp/mL (568-904000) and 3 pts developed cytopenia. In 11 pts HHV-6 was observed in bronchoalveolar lavage samples with a median of 502 cp/mL (57.50211); 9/11 pts had fever (5 bacterial and 1 fungal infection). In 16 pts (15 of them after allo-SCT, 9 with previous gut aGVHD) HHV-6 was also present in gastrointestinal biopsy (15 colorectal, 3 gastric) with a median of 5550 cp/mL (120-163800); in 4 cases HHV-6 was also found on plasma; 11/16 pts had diarrhoea. HHV-6 was found in cerebrospinal fluid in 3 pts (all within 30 days post allo-SCT); in 2/3 virus was also detected in peripheral blood; the median viral load was 19454 cp/mL (4506-39250); 3/5 pts experienced encephalitis showing confusion and anxiety, 1/3 seizure and 2/3 abnormal findings on brain MRI; all pts had fever and 1 skin rash. In all these cases HHV-6 was treated only when associated with potentially related clinical manifestations. Antiviral therapy was necessary in 23 pts (all received foscarnet).
except 3) and 13 of them solved the event. Among pts who experienced HHV-6 reactivation, 26/49 pts (53%) died. Conclusions: HHV6 reactivation is associated with high morbidity and mortality in hematological pts undergoing intensive treatment. Particularly, in pts who underwent allo-SCT HHV6 reactivation is associated with a poor outcome. A regular DNA monitoring is prospectively performed and a pre-emptive treatment is implemented in the setting of allo-SCT.

**1623**
CHARACTERISTICS AND OUTCOMES OF ADULT HAEMATOLOGY PATIENTS ADMITTED TO THE INTENSIVE CARE UNIT (ICU)
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Background. The role of intensive care support for haematology patients has historically been contentious. Aims. To profile patient characteristics and assess outcomes of adult haematology patients admitted to ICU. Methods: Retrospective audit of medical notes, laboratory records and Intensive Care National Audit and Research Centre (ICNARC) data for all adult haematology patients admitted to Belfast City Hospital ICU in 2009. Results: Twenty-one patients were admitted to ICU; mean age was 56-years (SD 12.5), 52% were male and 19 (82%) had a malignant diagnosis. The main indication for admission was neutropenic sepsis with associated organ impairment (n=18, 85%). ICU mortality was 48%. ICU survivors had lower acute physiology and chronic health evaluation (APACHE II) scores, and decreased requirements for invasive ventilation and inotropic support. Three and six-month mortality rates were 62% and 67% respectively. Of the post-six month survivors, one had relapsed, one had responding disease and five remained in remission. Two patients have subsequently undergone a reduced intensity conditioning transplant. Conclusion: A third of patients survived >6 months indicating that critically ill haematology patients may benefit from ICU admission, allowing progression to potentially curative therapies.

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<th>Table 1. Characteristics of ICU survivors and non survivors.</th>
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**1624**
BONE MARROW ASPIRATION DISCLOSES DISSEMINATED HISTOPLASMOSIS IN AN APPARENTLY IMMUNOCOMPETENT PATIENT
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Background. Histoplasma capsulatum is a dimorphic fungus endemic in the American continent, Africa and Asia. People acquire histoplasma through inhalation of spores from soils contaminated by bat or bird excrements. In Europe cases are usually imported and non-frequent. Disseminated histoplasmosis normally affects patients with immunodeficiency. We report on a non-immunocompromised patient who developed a disseminated histoplasmosis due to a reactivation of a latent infection acquired during a previous travel to America more than 10 years earlier. Case report: A 39 year-old woman, with no prior medical history of interest, HIV negative, developed twelve days before admission fever (39.6 ºC), vomiting, diarrhoea and nocturnal cough. Blood tests showed thrombocytopenia (platelets 59x10^9/L) and mild transaminase elevation as the only pathological findings. After admission, her condition worsened with the appearance of bilateral basal pulmonary infiltrates and persistent fever, irresistible to treatment with Levofloxacin and Metronidazole. An abdominal ultrasound scan showed hepato-splenomegaly only. She was moved to the intensive care unit due to progressive respiratory failure that required intubation with mechanical ventilation and noradrenaline perfusion. A bronchoalveolar lavage was performed, with negative results for mycobacteria, fungi, viruses, legionella and other bacteria. Doxycycline and Meropenem were added to the treatment. All microbiological tests were repeatedly negative (blood cultures, coprocultures, parasites in stools, serological tests for HIV, brucella, Epstein Barr virus, cytomegalovirus, coxiella burnetti, s. pneumoniae, hepatitis, salmonella, chlamydia and mycoplasma, H1N1 virus, clostridium difficile toxin and Mantoux test). Some days later, the patient’s clinical condition progressively improved, allowing the withdrawal of respiratory support, but the fever reappeared. The patient was transferred to an internal medicine ward, with progressive pancypaenia (hemoglobin 7 gr/dl, leukocytes 3.7x10^9/L and platelets 37x10^9/L). A bone marrow aspirate showed an increased number of macrophages, with the presence of round PAS-positive pseudo-capsulated intracellular microorganisms, suggestive of infection by histoplasma capsulatum. A bone marrow biopsy showed the same microorganisms and a PCR for histoplasma capsulatum confirmed the diagnosis. Bone marrow cultures for bacteria, fungi and mycobacteria were negative. The patient was initially treated with Itraconazole, but due to sub-optimal response the treatment was changed to Amphotericin B (lipidic complex) for three weeks, followed by oral Itraconazole. At discharge, the patient was afebrile, asymptomatic, and the histoplasma capsulatum PCR in peripheral blood was negative. The only pathological finding in the blood count was an inflammatory anaemia that subsided after a month. Although the patient had not travelled recently to endemic areas, she had been in Costa Rica 12 years before this episode. Conclusions: this case highlights the utility of bone marrow aspiration/biopsy in the diagnosis of disseminated histoplasmosis. It emphasizes the importance of a detailed inspection of bone marrow aspirates, with special focus on macrophages if an infection is suspected.

**1625**
PREDICTION OF PROGNOSIS FOR CHILDREN CARED IN INTENSIVE CARE UNIT AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION.
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Background. The pediatric index of mortality 2 (PIM-2) is a useful scoring system for prediction of prognosis in children with ICU support. The oncological pediatric risk of mortality score (O-PRISM) was also used for intensive cared children with hematopoietic stem cell transplantation (HSCT). Aims: Using both O-PRISM and PIM-2, we investigated risk factors, prognostic prediction tool, and survival for early detection of admission to intensive care unit (ICU). Methods: We reviewed retrospectively medical records of children cared in our institution ICU after HSCT between 2004 and 2010. There were excluded in patients died within 2 hours after moving to ICU. We used O-PRISM and PIM-2 for prediction of prognosis. We analyzed worst parameters in ICU by t-test, ANOVA, Cox-regression, multiple logistic regression, and receiver operating characteristics curve (ROC). Results: Fifty five out of 54 children were admitted to ICU on post-HSCT period. Non-malignant disease was 8 patients, and 16 children were transplanted with high risk disease
status. The source of stem cells were 14 matched sibling donor, 22 unrelated, 19 mediasternal donor. In ICU was 9 days (7 – 110). The reasons of admission to ICU were 32 pulmonary, 14 neurologic, and 9 hemodynamic events. Twenty seven children did not take care of mechanical ventilation. Six patients (11.1%) were survived after intensive care. The factors with discharge with survival were mental status (P=0.04), although there were FiO2, prothrombin time, potassium, pupil reflex in univariate analysis. In multiple logistic regression, there were significant factors with O2CO2 (P=0.028), O2PRISM (P=0.058), and PIM-2 (P=0.004) for prognosis. For prediction of prognosis, O2PRISM (P=0.019) was superior to PIM-2 (P=0.485) in intensive cared children after HSCT. Conclusion: O2PRISM following HSCT is more predictable scoring system in children with ICU support, and Glasgow coma scale and PaCO2 were more reliable prognostic factors for ICU admission.

1626
CENTRAL VENOUS CATHETER INFECTIONS IN HEMATOLOGICAL PATIENTS: REPORT FROM A SINGLE INSTITUTION
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Central venous catheters (CVCs) are currently used in hematological settings for intravenous therapy, stem cell transplant (SCT), blood products and fluids infusion. However, catheter-related bloodstream infections (CRBSIs) are an important cause of morbidity and mortality. Aims: The study aimed at retrospectively analyzing infectious complications in hematological patients. Patients and methods: One hundred fifty-five CVCs (100 Port-a-Cath, 57 external CVC), inserted from March 2001 to June 2010, have been analyzed. Patients with external CVC received chemotherapy for acute leukemia or autologous SCT for myeloma or lymphoma. Patients with port-a-cath systems, affected by lymphoma or myeloma, underwent less intensive chemotherapy. Results: Out of 57 external CVCs, 6 experienced infections: 4 sepsis (Paeruginosa), 1 skin tunnel infection (Paeruginosa and S.epidermidis), 1 endocarditis (Streptococcus oralis). Three fatal cases occurred in leukemic patients treated with intensive chemotherapy. Infections did not affect catheter survival. Performance status did not influence catheter infections, neither fever nor infections at the time of procedure. Percutaneous technique was significantly associated to CRBSI (p=0.028). CVC infections were more frequently related to left side insertion and to percutaneous technique (p=0.025). CRBSI was not statistically related to the type of immunosuppressive therapy (p=0.72). The underlying disease (p=0.05) and the type of therapy (p=0.02) were significantly associated with development of Paeruginosa infections. Patients with a neutrophil counts < 500/µl, for more than 10 days more frequently developed Pseudomonas infections (p=0.002). Out of 100 port-a-cath, sepsis occurred in 8 port-a-cath, 2 by S.epidermidis, and one by fungal infection. Overall, the infection rate per 1000cv days was 0.71 in external CVCs, and 0.65 in ports. Cumulative survival was mainly influenced by infectious complications (Log Rank and Breslow p<0.0001). Although not statistically different, patients who developed a CR-BSI had lower neutrophil counts at the time of insertion. Less intensive chemotherapy in patients with port did not influence CRBSIs. Summary/conclusions: Some studies reported that CRBSIs are more frequent in patients with malnutrition and unfavorable performance status. However, the presence of infections and unfavorable performance status did not influence CRBSIs in our patients with external CVC and port-a-cath. There are conflicting data from the literature upon the importance of neutrophil counts at the time of insertion: according to some authors, neutropenia is a risk factor for CVC-related infections. Our study did not report a correlation between neutrophil counts and infection development neither in external CVCs or in ports. Diagnosis and therapy did not influence CRBSIs rate because of heterogeneity and/or low number of patients. In this retrospective study were confirmed the data reported by others that the side and type of venous access devices for CRBSI and infections. Our results suggest that CRBSIs may promote colonization and biofilm formation, with higher infection risk, but we were not able to find this association in 157 CVCs. The present study contributes to underline the complex management of hematological patients and their particular susceptibility to CVC infections.

1627
PREVALENCE OF HEPATITIS C AMONG MULTI-TRANSFUSED THALASSEMIC PATIENTS IN THE SULTANATE OF OMAN: SINGLE CENTRE STUDY
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Background. Regular blood transfusion in thalassemia major is essential to maintain life. Risk of viral infections, especially hepatitis virus-es associated with blood transfusion, is a major concern. Screening of blood products for HIV, hepatitis B and C using the most sensitive methods have markedly decreased the risk of viral transmission. Aims. To evaluate the prevalence of hepatitis C in multi-transfused homozygous thalassemia patients in the Sultanate of Oman and to identify possible related risk factors. Methods. This is a retrospective chart review of all thalassemia major and interimedia patients (n=200) treated at the thalassemia Unit at Sultan Qaboos University Hospital in Oman. Relevant demographic and clinical characteristics were collected along with liver function tests, anti-HCV, and HCV RNA. Analyses were conducted using descriptive statistics. Results. Mean age of the patients was 28 ± 7 years ranging from 7 to 50 years. Eighty-one (41%) thalassemia patients were found to be HCV-Ab positive. HCV-Ab positive patients were significantly older compared to their HCV-Ab negative counterparts (28 versus 20 years; p<0.001). 11 patients (5.5%) have become sero-positive (PCR positive) after institutional donor screening. HCV-Ab positive patients were significantly more likely to be diabetic (p=0.023). CRBSI was not statistically related to the type of immunosuppressive therapy (p= 0.72). The underlying disease (p=0.03) and the type of treatment (p=0.023) were significantly associated with development of CRBSI. CVC infections were more frequently related to left side insertion and to percutaneous technique (p<0.0001). Out of 100 port-a-cath, sepsis occurred in 3 port-a-cath, 2 by P.aeruginosa and S.epidermidis, 1 endocarditis (P.aeruginosa and S.epidermidis), 1 skin tunnel infection (Paeruginosa and S.epidermidis), 1 endocarditis (Streptococcus oralis). Three fatal cases occurred in leukemic patients treated with intensive chemotherapy. Infections did not affect catheter survival. Performance status did not influence catheter infections, neither fever nor infections at the time of procedure. Percutaneous technique was significantly associated to CRBSI (p=0.028). CVC infections were more frequently related to left side insertion and to percutaneous technique (p=0.025). CRBSI was not statistically related to the type of immunosuppressive therapy (p=0.72). The underlying disease (p=0.05) and the type of therapy (p=0.029) were significantly associated with development of Paeruginosa infections. Patients with a neutrophil counts < 500/µl, for more than 10 days more frequently developed Pseudomonas infections (p=0.002). Out of 100 port-a-cath, sepsis occurred in 8 port-a-cath, 2 by S.epidermidis, and one by fungal infection. Overall, the infection rate per 1000cv days was 0.71 in external CVCs, and 0.65 in ports. Cumulative survival was mainly influenced by infectious complications (Log Rank and Breslow p<0.0001). Although not statistically different, patients who developed a CR-BSI had lower neutrophil counts at the time of insertion. Less intensive chemotherapy in patients with port did not influence CRBSIs. Summary/conclusions: Some studies reported that CRBSIs are more frequent in patients with malnutrition and unfavorable performance status. However, the presence of infections and unfavorable performance status did not influence CRBSIs in our patients with external CVC and port-a-cath. There are conflicting data from the literature upon the importance of neutrophil counts at the time of insertion: according to some authors, neutropenia is a risk factor for CVC-related infections. Our study did not report a correlation between neutrophil counts and infection development neither in external CVCs or in ports. Diagnosis and therapy did not influence CRBSIs rate because of heterogeneity and/or low number of patients. In this retrospective study were confirmed the data reported by others that the side and type of venous access devices for CRBSI and infections. Our results suggest that CRBSIs may promote colonization and biofilm formation, with higher infection risk, but we were not able to find this association in 157 CVCs. The present study contributes to underline the complex management of hematological patients and their particular susceptibility to CVC infections.

1628
TWO YEARS WITH PANDEMIC H1N1 2009 INFLUENZA A IN A TERTIARY HEMATOLOGY DEPARTMENT
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Background. Pandemic influenza A H1N1 2009 has been the prevailing influenza strain in Czech republic from autumn 2009 till March 2011. Severly immunocompromised patients with hematological malignancies and after stem cell transplantation are at increased risk of severe and dev-
and therapeutic approaches in hematology department. Immediate institution of aggressive treatment to early diagnosed cases in 2010/2011 season enabled recovery in all cases which contrasts with our early experiences from 2009/2010 season.

1629 CHARACTERISTICS AND RISK FACTORS FOR DEEP TISSUE ABDOMSSES IN A CONSECUTIVE COHORT OF PATIENTS WITH ACUTE MYELOID LEUKAEMIA: A NATIONAL POPULATION STUDY IN TAIWAN

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Background. Abscess may occur as an infectious manifestation in acute myeloid leukaemia (AML) patients. However, deep tissue abscess is more complicated since bleeding tendency was a major obstacle for diagnostic approach or surgical drainage in leukaemic patients. Aims. We focused on the prevalence of deep tissue abscess in patients with AML and the clinical characteristics and risk factors of AML patients developing deep tissue abscess. Methods. 5066 newly diagnosed patients from Jan 1997 to Jun 2006 were retrospectively analyzed from the Taiwan National Health Insurance Research Database in Taiwan. Deep tissue abscess was defined as abscess occurred at gastrointestinal tract, genitourinary tract, liver, deep neck, eye, central nervous system, mediastinal or pulmonary, and oral cavity in all AML patients after the diagnosis of AML. Eligible patients were sub-grouped as abscess group (n=765) and non-abscess group (n=4291). We determined the factors potentially associated with deep tissue abscess incidence. Results. The prevalence of all kinds of deep tissue abscesses in AML patients is 15.2%. The most predominant site is gastrointestinal tract abscess (n=290). The median time from AML diagnosis to deep tissue abscess is 151 days. 197 (25.6%) patients with deep tissue abscess have systemic infection within 30 days prior to the abscess diagnosis. Three independent risk factors were identified in predicting deep tissue abscess development in AML patients within 36 months after AML diagnosis. They are age less than 60 year-old (p<0.001, HR=1.871), male gender (p=0.004, HR=1.249), and secondary AML (p=0.01, HR=1.360). Risk stratification system was categorized according to the numbers of independent risk factors (0, 1, and more than 2 risk factors). The cumulative incidence of abscess within 36 months after AML diagnosis according to the risk stratification system was 0%, 9.4% and 66.5% (0 factors vs. 1 factor vs. more than 2 factors, respectively, p<0.001). Conclusions. Deep tissue abscess are not uncommon in AML patients. Patients who are male, older than 60 year-old and secondary AML warrant special attention since they are prone to have abscess after the diagnosis of AML.

1630 CIRRHOSIS ASSOCIATED WITH POOR OUTCOME IN ACUTE MYELOID LEUKAEMIA PATIENTS DEVELOPED LIVER ABDOMSSES: A NATIONAL POPULATION STUDY IN TAIWAN

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Background. Liver abscess following prolonged neutropenic periods during disease course has emerged as a major infectious complication for patients with acute myeloid leukaemia (AML). Previous report has showed that bleeding tendency was a major obstacle for diagnostic approach and surgical drainage in leukaemic patients. Aims. We focused on whether the surgical drainage procedure can prolong survival and determined factors associated with survival in AML patients developing liver abscess. Methods. We retrospectively reviewed data drawn from the Taiwan National Health Insurance Research Database which cover the entire civil of Taiwan. Cases of liver abscess (ICD-9 code 572.0) were defined as an intrahepatic infection with abscess formation occurring in all patients after the diagnosis of AML between Jan 1997 to Jun 2006. Amoebic liver abscess is excluded. We computed the impact of surgical drainage on 30 days survival after the diagnosis of liver abscess. We further examined whether other comorbidities increased the relative risk of death of 30 days after the diagnosis of abscess among patients with AML. RESTULS: The prevalence of liver abscess in all AML patients is 2.6% (n=132, 132/5066). Only 8 patients (61%) received surgical drainage. The surgical drainage did not improve the 30 days survival after liver abscess diagnosis (p = 0.016). Conclusions. Surgical drainage cannot improve the 30 days post-abscess survival in AML patients. AML patients with liver cirrhosis or ischemic heart disease have increased relative risk of death 30 days after liver abscess diagnosis.

1631 COMPARATIVE EVALUATION OF CELL DIFFERENTIAL IN BODY FLUIDS USING AUTOMATED HEMATOLOGY ANALYZER AND VISUAL MICROSCOPY

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Background. The new generation automated hematology analyzer Sysmex XE-5000 provides rapid body fluid analysis, including the differentiation of polymorphonuclear and mononuclear cells. The technology used is based on fluorescence flow cytometry. The precision is ensured due to extended counting in contrast with the visual microscopy. It is important to determine whether the fluid is a transudate or an exudate because it can be helpful in diagnosing the disease or condition present. Aim. The aim of this study was to evaluate the accuracy of Sysmex X5000 in determination of the cell differential in common body fluids. Methods. Body fluid (BF) samples were prospectively evaluated: pleural fluid, acistic fluid, continuous ambulatory peritoneal dialysis fluid, and synovial fluid. All fluids were collected in dipotassium ethylenediaminetetraacetic acid (K2EDTA) anticoagulated tubes. Differential counts were made by classical method, counting 200 cells under light microscopy on slides stained with May-Grünwald-Giemsa, and automatically with Sysmex X5000 without pre-treatment of the samples. Mesothelial, polymorphonuclear and mononuclear cells were differentiated. Results. Results for conventional cell categories compared excellent between Sysmex XE-5000 and visual microscopy. Strong correlation was observed between the manual and the automated method as for mesothelial, neutrophils and mononuclear cell differentiation (r=0.888, p<0.001, r=0.928, p<0.001, r=0.980, p<0.001, respectively). No extreme discrepancies, comparing the two methods, were noticed during measurement. Only in one sample the manual examination of 400 cells was needed in order to confirm the correspondence with the analyzer. Conclusion. In summary, our investigation revealed an excellent agreement between manual and automated differential of body fluid cells, indicating the reliability of the automated analyzer and therefore its utility in daily practice.

1632 FUNGAL INFECTION PROPHYLAXIS WITH POSACONAZOL IN ACUTE MYELOID LEUKAEMIA

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Invasive Fungal Infection (IFI) is a poor prognosis complication in Acute Myeloid Leukemia (AML). Patients with hematological malignancies and prolonged neutropenia are at high risk of IFI. Posaconazol is a 2nd generation triazole indicated in IFI prophylaxis (IDSA NCCN and EORTC guides). Aims: To evaluate the effectiveness of IFI prophylaxis with Posaconazol in profound neutropenia episodes in AML patients on intensive chemotherapy treatment. Methods: A retrospective study was carried out in two Spanish sites including AML patients treated with AraC based intensive chemotherapy receiving posaconazol as IFI prophylaxis. Effectiveness was defined as the occurrence of probable/proven IFI (EORTC criteria) and initiation of antifungal preventive therapy. Toxicity was evaluated with CTCAE v3.0 of NCI criteria. Results: In the period between January 2007 and December 2010, a total of 35 AML patients initiated IFI prophylaxis with oral Posaconazol 200 mg/8h. Mean age 57.3 years (26-76), male/female 19/16. Biweekly galactomannan antitgens (GmA) was analyzed. All patients were neutropenic. In 2 episodes were initiated because of positive GmAs and pathologic HTCAT; and 1 episode had pathologic HTCAT without GmA. There were 84 profound neutropenia episodes, with a mean duration of 19 days (10-58). The mean days of febrile neutropenia was 3 (0-7). Effectiveness: In 4 out of 84 episodes antifungal treatment with liposomal amphotericin was required: In 1 episode empiric therapy was initiated because of persistent fever with negative AGAs and no findings in HTCAT; in 2 episodes were initiated because of positive AGAs and pathologic HTCAT; and 1 episode had pathologic HTCAT without AGA. Toxicity: In 5 episodes Posaconazole prophylaxis was discontinued due to impossibility of oral administration: mucositis grade III-IV in
4 cases, and gastric hemorrhage in 1 case. No relevant renal or hepatic toxicity was observed. The incidence of probable/proven IFL of 2.5%, confirms Posaconazol as a high effective and well tolerated option for IFI prophylaxis, in patients with AML at high risk of fungal infection.

1633
GLYCOGEN PHOSPHORYLASE BB AS A POTENTIAL MARKER OF CARDIAC TOXICITY IN PATIENTS TREATED WITH ANTHRACYCLINES FOR ACUTE LEUKEMIA
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Background. Chemotherapy-induced cardiotoxicity remains an unresolved problem strongly impacting the quality of life and the overall survival of cancer patients. Anthracyclines represent the greatest risk for development of cardiotoxicity. From cardiac biomarkers, cardiac troponins have been recommended for monitoring of cardiotoxicity in oncology. Glycogen phosphorylase BB (GPBB) is among proposed biomarkers of cardiac injury with very limited experience in this context. Aims: To assess plasma GPBB concentrations in acute leukemia patients treated with anthracyline-based chemotherapy, to determine correlation with an chronic cardiotoxicity, and to compare plasma GPBB concentrations in acute leukemia patients with healthy blood donors. Methods: A total of 24 adult acute leukemia patients treated with 3-6 cycles of chemotherapy containing anthracyclines were studied. All patients had normal liver and renal functions during the study. Plasma concentrations of GPBB were measured at the diagnosis (before chemotherapy), after first chemotherapy with anthracyclines and circa 6 months after completion of treatment. The cut-off value for GPBB positivity was 10.00 µg/L as recommended by the manufacturer (Diagnostics, Germany). Twenty-four healthy blood donors were used as a control group. Results: Before chemotherapy, mean plasma GPBB concentration was 5.25 ± 5.81 µg/L, increased above the cut-off in 1 patient (4.2%). After first chemotherapy, mean GPBB was 6.61 ± 5.54 µg/L, positive in 7 (29.2%) patients. Six months after treatment, mean GPBB was 10.06 ± 11.41 µg/L, positive in 8 (33.3%) patients. The difference between GPBB concentrations before chemotherapy and 6 months after treatment were statistical significant (p < 0.05). The patient with GPBB positivity before chemotherapy (18.55 µg/L) had higher GPBB positivity in the subsequent samples (20.53 and 32.16 µg/L). Mean GPBB concentration in 24 healthy blood donors was 2.14 ± 0.28 µg/L (range 1.81 - 3.05), negative in all subjects. The differences in plasma GPBB concentrations between healthy blood donors and patients treated for acute leukemia were statistical significant (p < 0.05 in all cases). Conclusions: Our results suggest that GPBB could become a potential biomarker for detection of acute and chronic cardiotoxicity associated with anthracycline chemotherapy. Plasma GPBB concentrations within 6 months after treatment were significantly higher in comparison with baseline values. The predictive value for development of treatment-related cardiomyopathy in the future is not known and will be evaluated during the follow-up, as well as correlation with established biomarkers of cardiac toxicity and echocardiography. A larger prospective and multicenter study will be needed to define the potential role of GPBB and other proposed biomarkers of cardiac injury in the assessment of chemotherapy-induced cardiotoxicity.

1634
FIND - ‘FUNGAL INFECTION DATABASE’ - RETROSPECTIVE ANALYSIS OF INVASIVE ASPERGILLOSIS IN HEMATOONCOLOGICAL DEPARTMENTS IN CZECH AND SLOVAK REPUBLIC BETWEEN 2001-2009
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Background. Find - Aspergillus database (FIND) represents international database of invasive fungal infections in Czech and Slovak hematological departments. FIND - aspergillus covers all case of invasive aspergillosis (IA) in participating centers since 2001. Methods: The goal of our retrospective analysis was to evaluate incidence, early diagnostic procedures and effect of antifungal therapy in proven and probable IA that occurred in 9 institutions participating in FIND database between 2001-2009. We followed EORTC/MSG 2002 criteria in evaluation of IA diagnosis and therapy response. Results: 162 probable and 36 proven IA (91% isolated pulmonary IA, IPA) have been documented. Prolonged and profound neutropenia (63%) and long-term use of corticosteroids (81%) were identified as the major risk factors of IA. 161 cases (89%) had abnormality on pulmonary CT, however with non-specific infiltrates as the most frequent finding (49%). 74% pts. had consecutive positivity of serum-galactomannan (S-GM) (OD index > 0.5). 81% pts. with IPA and bronchoalveolar lavage (BAL) had positive GM in BAL. Budd (OD index > 0.5). In pts. with IPA only 9% BAL fluids and 21% spumum samples had positive microscopic result for filamentous fungi and 16% BAL fluids and 61% sputum samples had positive culture for Aspergillus spp. Primary mold active antifungal prophylaxis was used in 25% pts. - 15% iraconazole, 4% voriconazole (VORI), 5% posaconazol and others. Empiric antifungal therapy was used in 45% pts. with median of 5 days of administration before IA diagnosis (range: 2-44 days) - amphotericine B deoxycholate (C-AMB) was used in 31% of patients with empirical antifungal therapy, lipid-based AMB (LBA) in 24%, VORI in 18% and echinocandins (ECHINO) in 15%, respectively. The primary antifungal therapy of IA represents: in 58% of cases VORI, in 6% ECHINO, in 26% VORI-ECHINO, in 11% C-AMB and in 8% LBA. Overall RR to primary therapy of IA was 62% - VORI 62%, VORI+ECHINO 60%, C-AMB 52%, LBA 53%, ECHINO 20%. There was a statistically significant difference in overall RR to targeted therapy in pts. with neutrophil count < 0.1 and > 1.0 x10^9/L at the end of therapy (21% vs. 71%). The overall overall mortality rate was 57%, with 42% attributable to IA. Conclusions: On the basis of our analysis we confirm typical risk factors for IA and critical role of S-GM and CT for early diagnosis and prompt start of antifungal therapy of IA. A reasonable treatment response was achieved using VORI, VORI+ECHINO or LBA in primary therapy of IA. We have confirmed neutropenia at the end of antifungal therapy as the major predictive factor for therapeutic response.

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1635
CLINICAL CHARACTERISTICS AND OUTCOME OF INFLUENZA A (H1N1) INFECTION IN HEMATOLOGICAL PATIENTS DURING TWO CONSECUTIVE WINTER-SEASONS
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Background. Influenza A (H1N1) (IA-H1N1) infection usually results in mild respiratory symptoms in immunocompetent patients, whereas in immunocompromised patients might lead to severe complications and elevated mortality. Clinical characteristics of IA-H1N1 infection in patients with hematological neoplasms are not well described and need further investigation. Methods. Data from patients and infectious episodes of IA-H1N1 infection in hematological patients from a single institution were prospectively collected from November 2009 to February 2011 (two winter seasons). All patients with clinical suspicion of respiratory viral infection were diagnosed with IA-H1N1 real-time PCR analysis of nasopharyngeal aspirate or nasal swab. Results. A total of 25 patients were diagnosed with IA-H1N1 infection during the study period (11 in the 2009-10 season and 14 in the 2010-11 season). Median age was 66 years (range 17-89) Most frequent underlying diseases were non-Hodgkin lymphoma (n=9) and chronic lymphocytic leukemia (n=4). Five patients had received hematopoietic cell transplantation (2 allogeneic). All patients but one (pneumonia) presented with upper respiratory tract infection. Main clinical symptoms were fever (n=24, 96%), cough (n=16, 640 | haematologica | 2011; 96(s2)
64%) and rhinorrhea (n=8, 32%). All patients were treated with oseltamivir 150-75mg bid for a median of 5 days (range 3-20). A second RT-PCR determination of IA-H1N1 was performed in 9 patients with persistent clinical symptoms at the fifth day of treatment. Of them, four patients had persistent H1N1 positivity and were treated until negativity. Seventeen (68%) patients required hospitalization for a median of 5 days (range 1-40). Risk factors for hospitalization were low hemoglobin and platelet levels, and low oxygen saturation measured by pulse oximetry at diagnosis. We did not observe IA-H1N1-related deaths. Five patients (20%) had received prior IA-H1N1 vaccine. No differences in the severity of the infection (oxygen saturation at diagnosis, duration of respiratory symptoms, or need for hospital admission) were observed between vaccinated and non-vaccinated patients.

**Conclusions:** In patients with hematological diseases, clinical presentation and outcome of IA-H1N1 infection were similar to that described in immunocompetent patients. IA-H1N1 infection may occur in vaccinated patients and presents similar characteristics and outcome than in non-vaccinated patients.

**1636**

**NEW TECHNOLOGIES TO DEVELOP LONG-ACTING FILGRASTIM: GLYCOPEGYLATION OF r-METHUG-CSF AND ALBUMIN-FUSION OF NATURAL G-CSF SERVE A ONCE-PER-CYCLE FIXED DOSE STRATEGY TO PREVENT FEBRILE NEUTROPENIA**

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**Background:** Long-acting filgrastim offers the advantage of less dosing intervals when used for reduction of neutropenia and incidence of febrile neutropenia (FN) in patients treated with cytotoxic chemotherapy for malignancy. The target is a once-per-cycle fixed dose administration to prevent FN and being convenient for patients and their treating physicians. **Aims:** GlycoPEGylation and albumin-fusion were evaluated to serve as platform technologies to design a once-per-cycle fixed dose filgrastim. Methods: GlycoPEGylated r-HuG-CSF (GPG) was designed by attaching a 20 kD PEG to glycan at natural O-glycosylation site. A completely recombinant albumin fusion protein was developed by fusing natural G-CSF to human serum albumin through recombinant DNA technology. Pharmacokinetic and pharmacodynamic non-clinical and clinical studies were performed for both products. Once-per-cycle fixed dose approach for the prevention of chemotherapy induced FN has been investigated in clinical studies with pharmacokinetic, pharmacodynamic, efficacy and safety endpoints. Results: Compared to unmodified natural G-CSF, both glycoPEGylated r-HuG-CSF (GPG) and albumin-fused hu-G-CSF (AFG) have a prolonged circulating half-life. GPG and AFG show the expected pharmacologic activity of G-CSF in vivo, i.e. they stimulate neutrophil recovery in a dose-dependent way measured by an increase of absolute neutrophil count due to the induction of neutrophile pre-cursor proliferation. Efficacy and safety data obtained in clinical studies showed the expected profiles for a long-acting filgrastim. Summary / Conclusions: The platform technologies of glyco-PEGylation and albumin-fusion can be used effectively to obtain long-acting filgrastims and many other proteins. Pharmacokinetic and pharmacodynamic characteristics of GPG and of AFG show that these long-acting filgrastims are suitable for a once-per-cycle fixed dose use to prevent FN in patients treated with cytotoxic chemotherapy for their malignant tumor disease. The results for pharmacokinetics and pharmacodynamics for both products as well as the current efficacy and safety endpoints are as to be expected for a long-acting filgrastim supporting an efficient and safe once-per-cycle fixed dose administration.

**1637**

**THE EMERGENCE OF MUCORMYCOSES IN PATIENTS WITH HEMATO-ONCOLOGIC DISEASES**

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**Background:** Mucormycoses in patients with hematopoietic diseases are increasing in past few years, representing the third leading cause of invasive fungal infection in this patients following Aspergillus and Candida species. This increase may correspond to changes related to antifungal prophylaxis, particularly with the azoles, and the wide-spread use of antifungal agents. Historically, Mucormycoses presents itself as a fatal fungal infection, reaching in some series 90% mortality. **Aims and Methods:** We proceeded to the retrospective analysis of Mucormycoses at our centre in the last six years. Diagnosis was defined as probable or definite according to the EORTC/MSG criteria. Results: Mucormycoses were diagnostic in 5 patients, median age 54 years (range 20-78); 3 with Acute Myeloid Leukemia (4/5) and bone marrow aplasia (1/5). The diagnosis was verified in neutropenia (median 20 days, range 3-57 days) during induction (2/5), consolidation (2/5) and salvage therapy (1/5) of current chemotherapy protocols. Regarding the mode of presentation, 2/5 of cases were with pulmonary involvement and 3/5 with rhinosinus. Computed tomography was performed in all patients after prolonged febrile syndrome with symptoms suggestive of fungal infection and liposomal amphotericin B 5mg/kg/day was started empirically. Patients with rhinosinus involvement underwent surgical debridement and those with lung involvement underwent thoracic surgery (right lower lobe and upper left lobectomy). One patient required a second surgery with lobectomy and partial hepatectomy for liver involvement and diaphragmatic extension of fungal process. The historical study of surgical specimens confirmed the diagnosis of suspected Mucormycoses in all patients. Mycological culture was negative in all cases. Liposomal amphotericin B was maintained for a median of 50 days (15-80 days), followed by a period of secondary prophylaxis also with Liposomal amphotericin B in tapered low dose scheme, for a median of 3 months (range, 1-6 months). No mortality was attributable to fungal complication, the only death verified was due progression of underlying disease. Remaining patients had resolution of Mucormycoses and 3/4 completed the chemotherapy protocol designed to reach complete remission of hematopoietic malignancies. Conclusions: Although we had a mortality rate lower than that reported in other series of patients, this experience shows the challenge that Mucormycoses represents in patients with hematopoietic diseases. Clinical presentation is unspecific and diagnosis is difficult to establish. Early suspicion associated with high-dose antifungal therapy and aggressive surgical approach is the best option for treating these infections often fatal.

**1638**

**DEVELOPMENT OF RESISTANT INTESTINAL BACTERIA IN HEMATOLOGICAL PATIENTS WITH PROLONGED NEUTROPENIA WHILE ON LEVOFLOXACIN PROPHYLAXIS**

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**Background:** Levofoxacin prophylaxis (LP) is used to reduce the number of febrile episodes in neutropenic patients, although bacterial resistance is a matter of concern. We carried out a prospective study to know the impact of levofoxacin prophylaxis (500 mg o.d) over the intestinal flora with special emphasis in extended spectrum β-lactamase Enterobacteriaceae (ESBL-E) and ampicillin-resistant Enterococciaceae faecium (ARE). Material and methods: 17 patients diagnosed of leukaemia, lymphoma or multiple myeloma were receiving conventional chemotherapy or conditioning chemoradiotherapy before transplant, with an expected neutropenic time >10 days. We enrolled in this study. Patients received levofloxacin until the development of fever, when a beta-lactam was added empirically and levofloxacin withheld. Faecal samples at admission, during LP and while on beta-lactam therapy were seeded in ampicillin resistant E.faecium (ARE) and in ciprofloxacin (0.1mg/L) and cefotaxime (18-64μg/ml) susceptible (CP-E) and resistant Enterobacteriaceae (ESBL-E) and/or carbapenemase producing (CP-E) Enterobacteriaceae. Colonies were screened for ESBLs and carbapenemases. Clonal relatedness was studied by PFGE. Blood and urine cultures, obtained while the patient was febrile, were studied according to standard methods. Results: 220 faecal samples in 28 different neutropenia episodes were studied.
Mean duration of levofloxacin treatment was 10 days per episode. Percentage of patients colonized in the different study periods is shown in Table 1. Clonal analysis led to ESBL-E. The detection of four strains of E. coli Fq-resistant in the blood cultures of two patients, one of them being an exact clone of the one isolated in that same patient’s feces. No carbapenemase producing strain was detected. - AFE: ABE bacteriaemia was found in three patients (18%), all of them due to clone B (ST117) (the number of transfusion days ranged from 59% to 72% of the total isolations). 75% of the isolations were resistant to Levofloxacin, 88% exposed high level resistance to streptomycin and all of them remained susceptible to vancomycin, teicoplanin, daptomycin and linezolid. Conclusions: In the neutropenic patient under LP, a high faecal colonization by Fq-resistant Enterobacteriaceae and ARE was found, whilst ESBL-E was less noticeable. The levofloxacin-select- ed clones may cause ulcerator bacteriaemia.

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NOSOCOMIAL INFECTIONS IN ADULT HEMATOLOGY/ONCOLOGY PATIENTS
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Background: Hematology patients present an increased risk of nosocomial infections that vary in different populations and different institutions with considerable morbidity and mortality. Aims: The purpose of our study was to evaluate the frequency and patterns of nosocomial infections in hematology patients and to determine the prevalence of causative organisms and their antimicrobial sensitivity. Methods: A retrospective analysis was made in the patients admitted between April 2010 and January 2011 to the Department of Haematology of the First Propedeutic Department of Internal Medicine of University Hospital AHEPA. The 50 patients showed 240 admissions and 4002 inpatient days. The Centers for Disease Control and Prevention criteria were used as a standard definition for nosocomial infections. Results. The overall rate of nosocomial infections in hematology patients was 9.7 and in neutropenic patients 22.9 per 1000 patient-days respectively. Nosocomial fevers of unknown origin constituted 62.9% of the cases. The frequent sites of nosocomial infections were respiratory system (42.7%), the blood stream (25.3%), the urinary system (22.2%) and others (9.8%). The incidence of nosocomial infections was significantly higher during neutropenic days (P<0.001). Gram-negative organisms represented 71.2% of pathogens (Klebsiella 48.6%, Pseudomonas 35.7%, Acinetobacter 7.8%, E. coli 7.8%, and C. albicans 5.5%). Gram-positive organisms represented 28.8% of pathogens (Staphylococci 81.5%, Streptococci 18.5%). Positive cultures were more frequent in winter (November to March). Susceptibility of isolated organisms was relatively low (ampicillin/sulbactam 49.9%, amikacin 39.5%, imipenem 34.4%). Methicillin-resistant S. aureus and extended spectrum beta lactamase represented 50% and 65% of isolated S. aureus and Gram-negative organisms respectively. Multiresistant strains of Klebsiella and Pseudomonas were isolated from the patients. KPC-2 carbapenemase-producing Klebsiella strains were isolated from the patients. Conclusions. Respiratory infection and fever of unknown origin are the most common nosocomial infections in adult hematology patients with a higher risk during neutropenic days. Isolated organisms are multi-drug resistant, predominantly Gram-negative pathogens with a high incidence of extended spectrum beta lactamase, methicillin-resistant S. aureus, and carbapenem resistant organisms.

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RISK ASSESSMENT FOR BLOOD TRANSFUSION REQUIREMENT OF AT LEAST 3 UNITS DURING THE APLASTIC PHASE FOLLOWING CHEMOTHERAPY
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Background: Faced with the ongoing reduction in the number of blood donors, the clinical hematologist must often ask patient families for help in finding a compatible and persistent donor, detected in 59% of the patients and improved communication with the blood bank, allowing the clinical hematologist to order the appropriate amount of blood units necessary for the whole duration of the aplasia. Our passed experience was creat-
anti-Leb (4), anti-Cx (4), anti-c (3), anti-Jkab (3). None of the patients above was reported to develop any kind of immediate or later hemolytic reaction, despite the repetitive transfusions. **Conclusions.** The most frequent alloantibodies which are detected in the multitransfused patients of our hospital, are mainly versus the Kidd and the Rhesus antigenic systems of the red blood cells, and less versus the Kidd, Duffy, MNS, F and Lewis antigenic systems. Continual and persistent searching and early detection of these alloantibodies, and transfusion with red blood cell units which are negative for the analogue antigen, can offer the prevention in the severe reactions like the hemolytic episodes which worsen even more the bad general condition of the analogue patients.

1642

THE USEFULNESS AND DEMERIT OF THE EXPECTED ELEVATION VALUE OF SERUM ALBUMIN BEFORE REPLACEMENT THERAPY

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**Background and Aims:** Pharmaceutical albumin has been used excessively in Japan. In the 1980s, Japan consumed one third of the world supply. Although aiming to decrease dependence on albumin based treatments, Japan’s consumption of pharmaceutical albumin was 57,541 kg in 1999, which was 1.6 and 6.2 times more than that of the U.S.A. and U.K., respectively. Since then, the target elevation value of serum albumin was adopted in the Japanese Guideline for Blood Transfusion to determine the necessary dosage of pharmaceutical albumin for patients. The dosage is calculated by the formula, the necessary dosage (g) = the expected elevation value (g/dl) x amounts of whole plasma (dl) / 0.4. It contributed considerably to decrease consumption to 36,816 kg in 2009. On the other hand, the measurement of serum albumin is currently shifting from bromocresol green (BCG) assay to bromocresol purple (BCP) assay because the latter has a lower contamination other than pure albumin. In this study, the clinical importance of the target value of serum albumin was reevaluated for the BCP assay. **Methods and Results:** We analyzed the serum albumin value by BCP and BCG analyses in 251 cases, and examined the correlation between them. Y (BCP) = 1.09X (BCG) - 0.52 served as the standard curve from 2.5 g/dl to 2.3 g/dl in chronic diseases. However, the consumption of pharmaceutical albumin increased 25%. Extensive examination revealed the poorer correlation between them 6 months later. We analyzed a further 28 cases whose serum albumin levels were less than 3.0 g/dl with BCG assay. Y = 0.95X - 0.29 served as the new standard curve and the correlation of coefficient value (r) was 0.87. After we modified the target elevation value to 2.0 g/dl for chronic disease, the consumption of pharmaceutical albumin returned to the previous level of 6 month ago. We also analyzed the purpose of pharmaceutical albumin treatment for each patient, and the background of each case. A hundred and nineteen bedside cases treated with albumin replacement therapy were investigated. We divided these cases into 3 groups according to serum albumin level (z); 2.5 g/dl < z < 3.0 g/dl, 2.0 g/dl < z < 2.5 g/dl, and z < 2.0 g/dl. The coefficient value (r) was significantly poorer in lower serum albumin levels, and the serum albumin value did not elevate more than expected after pharmaceutical albumin treatment in such cases (p<0.05). **Conclusions.** The indication of pharmaceutical albumin varies and is complex especially in cases whose serum albumin level is lower. Target elevation value of serum albumin might be continuously effective in reducing the consumption of albumin. However, we have to understand the difficulty in estimating the correct clinical dosage of pharmaceutical albumin from laboratory data even if we adopted more advanced assay. Simultaneously, we need more information about efficacy on each background disease accompanying hypoalbuminemia.

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HOME CARE MANAGEMENT OF TRANSFUSIONS IN HEMATOLOGICAL PATIENTS

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**Background.** Red blood cell (RBC) and platelets (PLT) transfusion is one of the most challenging tasks in the home care management of patients affected by hematological disease (HD). Indeed the treatment of anemia or thrombocytopenia are essential part of the global management of most HD patients. In erythropoetin-failed patients or in those unsuitable for this option, RBC transfusions remain the only available measure with little evidence that they improve hematocrit. We present the unique effective treatment for symptomatic thrombocytopenia. **Aims.** To evaluate the management of RBC and PLT transfusions at home during the last two years (2009-2010). **Methods.** There were 266 pts (125 male) with a median age of 81 (20 - 95) years. Diagnosis was as follows: acute leukemia 51, multiple myeloma 34, lymphoma 55, myeloproliferative 30, myelos, myelodysplastic syndrome 68, solid tumor 25, other diagnosis 25. Patients were followed at home for a mean of 8,1 (1 - 24) months. Therapy with erythropoetin stimulating agents was used in 100 pts (38%). Results. Overall, 163 (61%) and 12 (5%) patients required RBC and PLT transfusions, respectively for a total of 2197 and 361 RBC and PLT units, respectively. RBC and PLT units monthly requirement in transfused pts were a median of 0.33 (0.04-8.7) and 1.83 (0.4-13.8), respectively. **Aims and MDS diagnosis correlated with RBC units requirement; AL diagnosis correlated with PLT units requirement. All transfusions were safely administered at home without any untoward effect. Conclusions. O	extsuperscript{2}C is a particularly important issue for hematological patients. With this regard, management of pts requiring multiple and repeated admissions to receive RBC or PLT transfusions may be a concern for the affected individual and for its family. Our experience demonstrated that the administration of RBC and PLT transfusion at home is a feasible, reliable and effective procedure in our patient, avoiding the social and economic costs due to an inappropriate removal from his domestic environment. In conclusion, in our experience domiciliary management of transfusions represented an important added value to home care program, allowing the best humanization of this procedure for our patients.
HOME BLOOD TRANSFUSIONS: THREE YEARS OF EXPERIENCE IN MYELODYSPLASTIC SYNDROMES

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Background. Transfusion-related acute lung injury (TRALI) is an under-reported complication of transfusion therapy. It is the third most common cause of transfusion-associated death. TRALI is characterized by noncardiogenic pulmonary edema. The presence of HLA antibodies in the donor or recipient plasma supports the diagnosis of TRALI. There is no specific treatment other than supportive measures. Case reports: Case 1: A 46 year old male with Acute Myeloid Leukemia treated with "3x7" scheme with pancytopenia and without blasts in the bone marrow received platelets and two packed red cell units transfusion on the 21st day of treatment. 4 hours after the transfusion, he presented acute respiratory distress and was transferred to the ICU. All other causes of pulmonary edema were ruled out and TRALI was suspected. The patient died shortly afterwards. HLA antibodies in the donor resulted positive. Case 2: A 81 year old female with myelodysplastic syndrome and severe aortic stenosis was admitted because of cardiac failure. She required five packed red cell units over the following five days. On the sixth admission day, platelets were transfused because of severe thrombocytopenia. Two hours after transfusion, she had chills, fever, hypotension, dyspnea, bivascular crackles and tachycardia. She was transferred to the ICU, starting oxygen by mask, steroids, and diuretics. The patient recovered over the next 72 hours. Positive anti-HLA antibodies were present in the donor. Conclusion: Endothelial cell damage and increased capillary permeability in TRALI are possibly caused by antibodies generated by the host against leukocyte antigens present in the blood products transfused, or by other substances that modify the biological response of the pulmonary bed neutrophils. The inflammatory reaction leads to pulmonary edema and respiratory failure. Antibodies against leukocyte antigens are present in 60-90% of patients with TRALI. There are wide variations in the reported incidence of TRALI. These discrepancies are probably explained by underdiagnosis, difference in diagnostic criteria and lack of standardized reporting. Joint efforts by international experts to standardize case definition may contribute to improve diagnosis. Conclusion: Our two cases illustrate TRALI as a relevant differential diagnosis in patients with sudden respiratory failure within six hours of transfusion. It is essential to notify immediately to the blood bank all potential TRALI cases, to ensure proper investigation of donor risk factors and to test for HLA antibodies.

COMPARATIVE EVALUATION OF THERAPEUTIC EFFICACY OF PHOTOCHEMICAL TREATED AND GAMMA-IRRADIATED PLATELET CONCENTRATES TRANSFUSED TO PATIENTS AFTER AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The maintaining of functional properties of blood components for transfusion is one of the important requirements for the processing methods. Gamma irradiation (GI) of apheresis platelet concentrates (A-PLT) is used for inactivation of allogenic T-lymphocytes to prevent
transfusion-associated graft versus host disease (TA-GvHD) in susceptible recipients. Photocatalytic treatment (PCT) of A-PLT with amotosalen and long-wavelength ultraviolet A (UVA) light has been developed to prevent transfusion complications, associated with pathogens and donors leukocytes. Aims. The aim is to compare Gled and PCTed A-PLT on the base of clinical markers of their functionality and viability. Methods: There were selected 50 A-PLT transfusions (15 transfusions of Gled and 15 transfusions of PCT) to be characterized on the following criteria: 1h posttransfusion count increment (1h CI) and 1h corrected count increment (1h CCI), 24h posttransfusion count increment (24h CI) and 24h corrected count increment (24h CCI), hemostatic efficacy and acute transfusion reactions. A-PLT were collected in 35% plasma and 65% platelet additive solution. PCT of A-PLT was performed with 150 µM amotosalen and 3,6 J/cm2 UVA light. GI dose of A-PLT was 25 Gy. The platelet content per transfusion dose was 2,5 to 3,5 x 1011. Quality control of all physicochemically treated A-PLT samples was performed by flow cytometry on the base of activation (CD62P) and apoptosis (Annexin V binding) markers. All treated A-PLT were ABO compatible and were transfused to thrombocytopenic patients after autologous haematopoietic stem cell transplantation during the first 48 hours after collection. Results: After informed written consent was obtained, 12 patients were included in the study, each patient received 1 to 4 A-PLT transfusions. The mean pretransfusion platelet count was 19,2±5,5 x109/L. The average 1h CI in the group received PCTed A-PLT was 25,7±7,49 x109/L. There were no observed differences in patients groups with transfusions of PCTed and with Gled A-PLT: 1h CI in the group received Gled A-PLT was 23,8±5,22 x109/L. The mean 1h CCI was similar in both groups: 19,4±7,2 x109/L for the group received PCTed A-PLT and 15,3±5,6 x109/L for the group received Gled A-PLT. The mean 24h CI was 12,4±5,6 x109/L in PCT group, 24h CI for GI group was on the same level - 12,7±5,1 x109/L. There was no statistically significant difference (P>0,05) between the average 24h CCI for PCT and GI groups: 24h CCI was 9,2±4,6 and 8,2±3,6 x109/L respectively. All transfusions had adequate hemostatic efficacy. No episode of pretransfusion reactions were observed in these groups. Summary/conclusions: The results of 1h CI and CCI, 24h CI and CCI were acceptable according to the European recommendations and to international guidelines. The physicochemically treatment of A-PLT was not lead to decreasing of therapeutic efficacy of A-PLT. PCTed and Gled A-PLT were demonstrated the identical clinical markers of their functionality and viability. The PCT of A-PLT prevent a broad spectrum of transfusions associated risks that is why this method is preferable for patients after autologous haematopoietic stem cell transplantation.

1649 DIFFERENT METHODS FOR DETERMINATION OF LOW PLATELET COUNTS: THE REASON FOR POSSIBLE INAPPROPRIATE PLATELET TRANSFUSION

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Background. It is known that the use of available hematology analyzers is based on two basic principles: electronic impedance (IMP) and optical light scatter analysis (OPT). Both methods may result in some inaccuracies in PLT measurement, which can be caused by interference from cells or materials of a similar size to the PLTs. Therefore, immunophenotyping methodology is proposed as a new reference method, using monoclonal antibody specific to a cluster of differentiation common to all platelets. Aims. We decided to evaluate low PLT counts by IMP and OPT methods and to compare them with the immuno plat CD61 measurement by flow-cytometry. We also examined the possibility of inappropriate PLT transfusion resulting from an inaccurate PLT count. Methods. We analyzed consecutive blood samples of patients with acute myeloid leukemia and OPT PLT counts of less than 50x109/L. Also, we compared the number of prophyllactic PLT transfusion indications (threshold of 20 x 109/L) according to the PLT counts determined by the OPT and IMP methods with the number of prophyllactic PLT transfusion indications according to CD61 method. In our study we used Cell-Dyn Sapphire (Abbott - of great A-hr) to be characterized on the hematologic analyzer measured the PLT count by three methods: OPT, IMP, and CD61 methods using monoclonal antibody directed against glycoprotein Il差异 (CD61). Results. We collected 44 samples. The mean ± SD values of the PLT counts were 17x109/L, 16x109/L, and 21x109/L for the CD61, OPT, and IMP methods, respectively. The correlation of the OPT method when compared with the CD61 method was very strong (r = 0.872; P<0.001) and R2 was 0.761. The correlation of the IMP method when compared with the CD61 method was weaker (r = 0.686; P<0.001) and the R2 was 0.470. In the bias analysis, the IMP method (but not OPT) showed higher PLT counts when compared with the CD61 method (mean of difference 4.57±10x9/L, p=0.001 and 0.72±10x9/L, p>0.05, respectively). We saw overtransfusion in 29.5% of cases and undertransfusion in 2.3% of cases (p=0.002; McNemar’s test) when we selected a threshold of 20x109/L with the IMP method. Conclusions. PLT counts determined by the IMP methods showed some disagreement when compared with the CD61 and OPT methods. This disagreement caused both PLT undertransfusion and overtransfusion.

1650 POSITIONAL PARAMETERS FOR THE DETECTION OF VITAMIN B12 AND FOLATE DEFICIENCIES

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Background. Classical flow charts used widely for the diagnostic approach of anemia due to Vitamin B12 (B12) deficiency or anemia due folate deficiency includes the Mean cell Volume of the red blood cells (MCV) as one of the key tests for the suspicion of these diseases and differential diagnosis of anemia. Only around half of the patients with B12 deficiency or folate deficiency have high MCV, in many of the situations because the coexistence of other causes of anemia. The Coulter LH 780 hematology analyzer (Beckman Coulter) has the ability to measure specific parameters of neutrophil and monocyte populations like mean and standard deviation (SD) of cell volume (MVI,SDVI), conductivity (MCL,5DCI), and light scatter (MFI,5DSI). These so-called positional parameters can detect morphologic changes in neutrophil and monocyte population. Using VCS parameters we investigated the correlation between megaloblastic neutrophils and monocytes in B12 and/or folate deficiencies.
THE STRUCTURAL PARAMETERS NEUT-X AND NEUT-Y IN MEGALOBLASTIC ANEMIA
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Background. NEUT-X and NEUT-Y are new structural parameters determined by the Sysmex XT-2000i analyzer (provided by service data). NEUT-X is the mean value of the side scatter diffraction of the neutrophil population and represents the structure of the neutrophils, while NEUT-Y is the mean value of the fluorescence measurement. Low values of NEUT-X and NEUT-Y reflect neutrophil dysplasia in myelodysplastic syndromes. Aims. The aim of this study is to evaluate these new parameters in patients with megaloblastic anemia (MA) due to vitamin B12 deficiency. Methods. Blood samples from 62 normal healthy subjects - controls (normal results of blood count and blood smear) and from 40 patients with MA (hemoglobin < 12.0 g/dl, serum vitamin B12 levels < 120 pg/ml, determined by Access, Beckman Coulter analyzer, and microscopical hypersegmentation of neutrophils) were performed with Sysmex XT-2000i analyzer. Statistical analysis: Student’s t-test and Pearson correlation were applied. Values of P < 0.05 were considered to indicate statistical significance. Results: Patients with MA have statistically significant higher values of NEUT-X than normal controls, but there was no difference in NEUT-Y values (Table). In patients with MA, NEUT-X values correlate negatively, in a statistically significant degree, with vitamin B12 deficiency (r=-0.577, P=0.001). Conclusions. The increased NEUT-X value (an index of the structure of neutrophils) observed in patients with MA, reflects the presence of hypersegmented neutrophils and could help with the diagnosis of megaloblastic anemia. The laboratory cytologist should consider above which threshold of NEUT-X value, a microscopic blood film review is routinely necessary.

EXTENDED LEUKOCYTE DIFFERENTIAL OF LEUKOPENIC SAMPLES USING COMBINATION OF AUTOMATIC CELL ANALYZER AND 5-COLOR 6-ANTIBODIES FLOW CYTOMETRY
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Background. The performance of the manual differential on leukopenic samples has very poor precision due to insufficient number of counted cells. Frequently, it is very difficult to classify cells and time consuming also. Recently, the HematoFlow method has been introduced as a combination of automatic cell analyzer and CytoDiff® 5-colors 6-antibodies flow cytometry. Aims. We evaluated the usefulness of HematoFlow method in leukocyte differential of leukopenic samples. Methods. Leukopenic blood samples were analyzed by flow cytometry using premixed reagent for leukocyte differential and no wash mode(CytoDiff, Beckman Coulter). HematoFlow results were selected or calculated using automatic blood cell analyzer(DxH800, Beckman Coulter) results and CytoDiff results. Manual differential counting was duplicated including a hematopathologist(reference count) and 50 to 100 cells were counted. Immature granulocytes(IG) of manual count was sum of metamyelocytes, myelocytes and promyelocytes. IG was calculated by subtracting the DxH800 eosinophil count from sum of CytoDiff IG and eosinophil count. If basophil and nonB nonT blasts populations were not well separated, differential was performed using median fluorescence level of 3.5 using CD2+CRTH2. Statistical analysis was performed using Fisher’s P test for analysis of precision and Pearson correlation test for correlation analysis. The study was approved by IRB. Results. The proportion of adjusting gates in CytoDiff was 19 out of 247 cases(7.7%) due to huge debris contamination(2 cases) and incomplete separation of basophils and blasts. The precision of HematoFlow was superior to manual differential in counting of 5 leukocyte subpopulations, IG and blasts(Table 1). Especially, the eosinophil count by duplicated manual count revealed 5.4±5.8% difference and by HematoFlow revealed only 1.24±2.70% difference. The blast was found in 46 samples and 17 samples showed blasts only in one manual count(37%), but all cases showed blasts in both HematoFlow counts. The manual blast count showed 1.7±6.6% difference and by HematoFlow only 0.65±1.99% difference. The eosinophil count of DxH800 showed best correlation to reference count. All data analysis is summarized in Table 1. Conclusions Leukocyte differential in leukopenic samples using HematoFlow is much more reproducible and accurate than manual differential. Especially, detection of leukemic blasts is much more reliable than manual differential. In conclusion, HematoFlow is very useful in differential counting of leukopenic samples.

OUTCOME AND RELAPSE RISKS OF THROMBOTIC THROMBOCYTOPENIC PURPURA: AN EGYPTIAN EXPERIENCE
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Background: Thrombotic thrombocytopenic purpura [TTP] whether idiopathic or secondary is a rare but life threatening condition. Plasma exchange [Px] has decreased significantly the mortality from the disease but a substantial proportion of patients relapse. We describe the clinical spectrum and response to treatment and explore the risks for relapse in our cohort of patients. Patients and Methods: Patients treated for TTP at the Clinical Hematology Unit of the Department of Internal Medicine of Cairo University Egypt, between 2000 and 2008 were identified. Complete demographic and clinical data, laboratory results, treatment modalities and outcome data were collected and analyzed. The follow-up duration was 24 months. ADAMTS13 and its antibody were assayed for idiopathic patients admitted after June, 2007. Results: A total of 30 patients; 13 males (43%) and 17 females (57%) with a median age of 42 years were treated for 46 episodes of TTP. The median duration of disease onset-to-diagnosis for the first episode was 7 days. 23 patients (76.66%) were diagnosed as idiopathic primary and 7 patients (23.33%) were secondary TTP. Four patients died during the first 24 hours. Out of the 26 patients followed beyond 24 hours, 22 patients (84.6%) achieved remission with an average of 7.5 Pxs sessions, 13 of whom achieved a sustained remission (50%) whereas 4 patients (15.3%) were refractory. Nine patients relapsed thereafter (total 25 relapses) (mean 2.7 per patient) with an average of 9 Pxs sessions to achieve a subsequent remission. The 24 months overall survival was 80%. Initial low platelet count and high LDH were the only two statistically significant relapse predictors. Conclusions: The current results are concomiring to the reported literature of the outcome of TTP. The very early mortality due to late referral highlights the need of education and awareness about the disease among primary health care providers.
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MYH9-RELATED PLATELET DISORDERS: A CHALLENGE IN LABORATORY MEDICINE - CASE REPORT

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Myosin heavy chain 9 (MYH9) - related platelet disorders belong to group of inherited thrombocytopenias. As a result of MYH9 gene mutation premature release of platelets from bone marrow is happening. Macrothrombocytopenia is present at birth and the only therapy that increases platelet count is transfusion of platelet concentrates. Considering the main characteristics of MYH9- syndrome, thrombocytopenia with very high MPV, laboratories should give precise platelet count, in order to avoid risk of inappropriate treatment. Impedance cell counters do not recognize giant platelets and, therefore, underestimate both platelet count and platelet volume, so manual assessment is required. Mean of this work is to establish a modern approach of platelet counting, using immuno-determination of superficial antigen CD61, as the most accurate diagnostic tool. The patient, 25-year old male, with MYH9 syndrome, confirmed clinically by presence of macrothrombocytopenia and inclusion Döhle’s bodies in granulocytes, was admitted to clinic for digestive surgery due to appendectomy. During routine laboratory investigation platelet counts, done by flow-cytometer Sapphire, Abbott Diagnostics, showed discrepancy in impedance and optical values (21 and 37 x10^9/L, respectively). Because of very large MPV (15,1 fl) and the lack of experience in such atypical thrombocytopenias, presumption was that CD61 will give the most precise platelet count and this count was the highest (49x10^9/L), which was confirmed microscopically. Not underestimated the vigor of impedance principle, immuno-platelet counting, as a reference method, even in this rare clinical cases proves its strength for the best platelet enumeration.

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THROMBOTIC COMPLICATIONS IN PATIENTS WITH ADULT IMMUNE THROMBOCYTOPENIC PURPURA

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Background. Immune thrombocytopenic purpura (ITP) is autoimmune disease characterized by platelet destruction and decreased platelet production. Occasionally in ITP a life-threatening hypercoagulable state develops after splenectomy. Also there are reports of thrombotic events after management of ITP with intravenous immunoglobulin (IVlg). We present two patients, the first with thrombotic complications after splenectomy as a result continuous platelet activation and normalization of platelet number and the second patient developed acute myocardial infarction 5 days after finishing 5-day therapy with IVlg. Case 1. A 53-year-old woman was diagnosed with ITP in February 2007. She had not other thrombotic risk factors. She was treated with corticosteroids and IVlg. The alternative treatment can also be anti-coagulant therapy. The patient, 25-year old male, with MYH9 syndrome, confirmed clinically by presence of macrothrombocytopenia and inclusion Döhle’s bodies in granulocytes, was admitted to clinic for digestive surgery due to appendectomy. During routine laboratory investigation platelet counts, done by flow-cytometer Sapphire, Abbott Diagnostics, showed discrepancy in impedance and optical values (21 and 37 x10^9/L, respectively). Because of very large MPV (15,1 fl) and the lack of experience in such atypical thrombocytopenias, presumption was that CD61 will give the most precise platelet count and this count was the highest (49x10^9/L), which was confirmed microscopically. Not underestimated the vigor of impedance principle, immuno-platelet counting, as a reference method, even in this rare clinical cases proves its strength for the best platelet enumeration.

Results: in the index cohort, out of 168 smokers with chronic leukocytosis, 13 presented, in addition, a high platelet count while in the control cohort, out of 60 smokers with a high leukocyte count, 8 presented with thrombocytosis. There were no major differences between both cohorts (Table). In particular, the incidence of thrombocytosis in patients with tobacco-induced leukocytosis was similar (7.7 vs 11.7%, p= 0.4). The slightly lower platelet count in the control cohort may be related to lower normal values in the control laboratory. Overall, the thrombocytosis was moderate and did not vary substantially during a protracted follow-up (median FU: 72 months), and was not associated with major arterial or cardiac events. Conclusions: although this study provides no direct evidence that thrombocytosis was induced by smoking, its unexpectedly high prevalence in persons with smoking-associated leukocytosis suggests a causal relationship with smoking, especially since similar incidence and characteristics were observed in 2 independent cohorts. Smoking-associated thrombocytosis should be considered in patients with otherwise unexplained long-standing, moderate, and stable thrombocytosis, associated with leukocytosis. The clinical course of this condition is benign. Such patients should not be submitted to aggressive or expensive investigations.

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CHRONIC ITP - CASE REPORTS OF THE TREATMENT BY TROMBOPOETIN ANALOGUES IN TWO CHILDREN

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Background. Immune thrombocytopenic purpura (ITP) is a common acquired bleeding disorder in children characterized by skin and mucosal bleeding. There is an isolated thrombocytopenia caused by antithrombocytic antibodies. The standard treatment is corticosteroids and intravenous immunoglobulin. The alternative treatment can also be anti-
CD20 antibodies and other immune suppression regimens. The splenectomy would be considered if the above-mentioned treatment fails, although we always try to avoid splenectomy in very young children because of possible severe complications such as post-splenectomy sepsis. This was the reason for using thrombopoietin analogues in two girls under the age of eight years, presented on this poster. Aims. To assess the efficacy of treatment with romiplostim in two children with chronic ITP Methods and Results. In the first patient neither intravenous immunoglobulin nor corticosteroids in dose up to 2mg/kg lead to a sufficient and lasting response. The treatment with antiCD 20 antibodies induced remission for just 5 months. Then platelets decreased again after a viral infection. In the second patient, corticosteroids induced remission only for a few days and after intravenous immunoglobulin the remission lasted only for 5 weeks. Moreover over the patient on repeated severe allergic reaction to intravenous immunoglobulin. Both of our patients fulfilled the criteria of chronic ITP and we offered them and their families treatment with romiplostim with the dose scheme based on data published by Buchanan et al (ASH abstract 680, 2009). Since our patients have been commenced on romiplostim, the platelet count is stable between 100-200x109/L. Conclusion. Although the prognosis in the patient with chronic ITP is uncertain and the treatment options may not guarantee permanent remission, the alternative treatment with thrombopoietin analogues induced long-term remission in our patients. This treatment also might help to postpone or avoid splenectomy in our two patients and thus improve their quality of life.

1658 CASE STUDY OF SEVERE THROMBOCYTOPENIA AND ASSOCIATION WITH THE COURSE AND FINAL OUTCOME OF THE DISEASE A RURAL HOSPITAL OF GREECE
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Background: Aims. To study the severe thrombocytopenia (PLT <50.000) which is a frequent problem in hospitalized patients, a causal association with various diseases and demonstrate the important role it plays in the disease course and outcome. Material-Methods. We studied retrospectively 1250 records of patients. Results. There were 52 patients (19 men and 33 women) with a platelet count 4000-5000/L. Of these 28 (72%) experienced major bleeding disorders. The average age for men was 66 years for women 71. Causes identified: - In 12 cases of malignant disease with chemotherapy onward (37.5%) In 6 cases of malignant hematological diseases (18.8%) In 6 cases of hepatic cirrhosis (18.8%) In 4 cases of sepsis (3.8%) In 3 cases of idiopathic thrombocytopenic purpura (9.4%) 1 case (3%) reptile bites Despite appropriate therapeutic treatment in 7 cases (22%) death occurred as a consequence of disseminated intravascular coagulation. Summary-Conclusions: 1. Most common cause of thrombocytopenia is a malignant disease after chemotherapy, followed by malignant hematological disease and followed by hepatic cirrhosis. 2. The profound thrombocytopenia is a serious prognostic sign and threatening situation for life itself, despite the appropriate measures. 3. Bleeding disorders occur at a low price in a large percentage of PLT. Attention, therefore, in these cases to the attention of all.

1659 RELAPSES AT THE PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA (ITP) TREATED AS FIRST-LINE THERAPY WITH HIGH DOSE DEXAMETHASONE VERSUS STANDARD STEROID THERAPY
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Background. Immune Thrombocytopenic Purpura (ITP) is an acquired disease characterized by an immunological peripheral platelet destruction, with a chronic evolution at adult patients. Glucocorticoids are the gold standard in the evaluation of platelet disorders. Nevertheless, there is a lack of consensus about the diagnostic tests concerning platelet function disorders. The abnormal platelet morphology was found in women because of the menstrual cycle and the haemostatic challenge of childbirths. 13 were diagnosed as disorders of platelet secretion and signal transduction (8 women/5 men), 1 as thrombasthenia Clanzmann, 1 as aspirin like defect, 4 due to antiphospholipid antibody testing, one had abnormal aggregation to collagen and one abnormal aggregation to ADP. The use of PFA-100 in our patients showed that among 16 patients tested, 3 had normal values but 5 of them had abnormal aggregation tests and diagnosed as disorders of platelet secretion and signal transduction. Among the other 8 patients with abnormal prolonged PFA-100, 4 were found to have normal aggregation tests, 3 were diagnosed as disorders of platelet secretion and signal transduction and one was due to antiphospholipid antibody testing. The abnormal platelet morphology was found in 4 out of the 13 patients diagnosed as disorders of platelet secretion and signal transduction. Platelet nucleotides release measurement was not feasible. The hemorrhagic history of the 13 patients who were diagnosed as disorders of platelet secretion and signal transduction was serious menorrhagia and bruises in the 8 women and the excessive bleeding following surgical and dental procedures in the 5 male patients. The aggregation tests give a lot of information about various platelet disorders as seen in our patients. Normal values of PFA-100 cannot exclude the diagnosis of a platelet function disorder as found in 5 of our patients, though the number of patients is limited. In literature and clinical practice there is a lack of information about the diagnostic tests concerning the platelet function disorders. According to our findings, the aggregation tests seem to remain the gold standard in the evaluation of platelet disorders.

1660 HEREDITARY PLATELET FUNCTION DISORDER INVESTIGATION; ONE YEAR RETROSPECTIVE IN THE HAEMOPHILIA CENTRE OF NORTHERN GREECE
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Hereditary platelet function disorders constitute a rare cause of symptomatic bleeding. These disorders are heterogeneous in clinical expression and laboratory evaluation. The accurate measurement of the platelet function and the identification of a congenital platelet disorder is a complex, includes different assays and the repetition of testing. 55 patients (42 women/13 men) were referred for platelet function evaluation due to personal and/or family bleeding tendency (51) and 4 to check the antiplatelet therapy. They all manifested symptoms of excessive mucocutaneous bleeding such as easy bruising after minor surgery or dental procedures. Patients with prolonged of PT, aPTT and TT were excluded and they were investigated for coagulation factors deficiencies. All patients were tested for Von Willebrand’s Disease. The platelet function investigation consisted of 1) the platelet count number and the 2) blood film report for white cell inclusions and platelet size, 3) collagen stimulation with platelet rich plasma with light transmission aggregometry (LTG) by the use of 5 agonists (arachidonic acid, ADP, adrenaline, collagen, ristocetin), and the 4) global test of haemostasis by PFA-100. The LTG was found normal in 34 patients (61.2%) and 21 patients (38.8%) had abnormal LTG. 14 patients among them (66.6%) were women as expected, since these disorders are more commonly diagnosed in women because of the menstrual cycle and the haemostatic challenge of childbirths. 13 were diagnosed as disorders of platelet secretion and signal transduction (8 women/5 men), 1 as thrombasthenia Clanzmann, 1 as aspirin like defect, 4 due to antiphospholipid antibody testing, one had abnormal aggregation to collagen and one abnormal aggregation to ADP. The use of PFA-100 in our patients showed that among 16 patients tested, 3 had normal values but 5 of them had abnormal aggregation tests and diagnosed as disorders of platelet secretion and signal transduction. Among the other 8 patients with abnormal prolonged PFA-100, 4 were found to have normal aggregation tests, 3 were diagnosed as disorders of platelet secretion and signal transduction and one was due to antiphospholipid antibody testing. The abnormal platelet morphology was found in 4 out of the 13 patients diagnosed as disorders of platelet secretion and signal transduction. Platelet nucleotides release measurement was not feasible. The hemorrhagic history of the 13 patients who were diagnosed as disorders of platelet secretion and signal transduction was serious menorrhagia and bruises in the 8 women and the excessive bleeding following surgical and dental procedures in the 5 male patients. The aggregation tests give a lot of information about various platelet disorders as seen in our patients. Normal values of PFA-100 cannot exclude the diagnosis of a platelet function disorder as found in 5 of our patients, though the number of patients is limited. In literature and clinical practice there is a lack of information about the diagnostic tests concerning the platelet function disorders. According to our findings, the aggregation tests seem to remain the gold standard in the evaluation of platelet disorders.

1661 EFFICACY AND SAFETY OF SIX DOSES OF RITUXIMAB (375MG/M²) IN TREATMENT OF CHRONIC REFRACTORY IMMUNE THROMBOCYTOPENIC PURPURA AN EXPERIENCE FROM QATAR
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Rituximab is a potential treatment for patients with chronic ITP (less than 10x10^9/L), partial response (PR) or if platelet count was higher than 5x10^9/L and failure if platelet count was lower than 5x10^9/L. At the end of glucocorticoids therapy, 15 patients had CR, 4 patients PR and 4 patients presented failure response, needed other therapies (vincristine, splenectomy). The median period of observation at responding patients was of 9,8 months. Seven patients of 19 responders received 4 of them initial treated with standard steroid therapy and 3 treated with HD-DXM needed other therapy (2 vincristine, 4 splenectomy, 1 eltrombopag). Conclusion: our study didn’t reveal major differences between relapses at the patients with ITP treated with HD-DXM versus standard steroid therapy.
Background. ITP is an autoimmune disorder leading to premature platelet destruction and persistent thrombocytopenia. Treatment is generally not recommended until the platelet count is <30,000/µL, bleeding occurs, or there are other predisposing co morbid conditions. The goal of treatment is to raise the platelet count to a hemostatically safe level. Different doses of Rituximab were tried 1 Gram fixed dose day 1 and day 15 (Rheumatoid arthritis like regimen), or 375mg/m²/week for four weeks and 100mg/week for four weeks but nobody has tried 375mg/m²/week for six weeks. Aims: to evaluate the efficacy and safety of 6 doses of Rituximab 375mg/m², in treatment of patients with chronic refractory thrombocytopenia. Patients and Methods. From our retrospectively collected data of 14 patients diagnosed with chronic refractory thrombocytopenia between the January 2007 and January 2011 in AL-Amal hematology/oncology centre with mean follow up of 27 months all of them failed, steroid as first line therapy as well as IVIG, and 4 of them underwent splenectomy with no response, 8 were males and 6 were females with a mean age of 52 year, all of them had platelets count of less than 10,000 on presentation. Each of them received Rituximab 375mg/m² weekly for six doses and followed initial with weekly CBC for 3months and then every two months. Results: two patients did not achieve any response one is male and the other is female. Two patients achieved partial response platelets count more than 50,000 and less than 100,000 for more than six month and 12 achieved complete response defined as platelets count more than 100,000 for more than one year, with mean complete response of 28 months, none of them developed grade 2 or more infection as per WHO toxicity scale, and none of them developed progressive multifocal leucoencephalopathy. Conclusion: We concluded that six doses rituximab are safe effective and durable in patients with chronic refractory ITP keeping in mind the small number of patients further studies are needed to confirm these results.

1662 TIME-RELATED CHANGES OF THE SENSITIVITY TO ANTI-PLATELET THERAPY IN PATIENTS WITH ACUTE CORONARY SYNDROME AFTER PERCUTANEOUS CORONARY INTERVENTION
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Study population. 37 patients (pts), 26 male, one month after an episode of acute myocardial infarction treated by percutaneous coronary intervention (PCI) with stent placement. All pts were receiving dual anti-platelet therapy (ASA - 75 mg/pd and clopidogrel - initial 600 mg, next 75 mg/pd) for at least one month before entering into the study. During the second and third year of observation all pts were on ASA 75-150 mg/pd. Methods: The platelet reactivity was assessed on a basic of closure time (CT) in platelet function analyzer EPFA100 (Dade Behring). CT was measured on the 30 th day and 36 months after PCI. ASA resistance was defined as a normal collagen/epinephrine CT (<165 s) despite ASA treatment (compliance proved by a diminished intraplatelet mylonyldialdehyde [MDA] concentration).

Results. During 3 - years follow-up changes of ASA resistance status were observed in 14 pts - 10 from “responsive” to “resistant” and in 4 pts from “resistant” to “responsive” status. In 23 pts there were no changes of ASA resistance status. The results of CT in 30 th day and 36 months after PCI were presented on the figure. Conclusions: During 36 months follow-up the changes of ASA resistance status (assessed using PFA-100) were observed in 33% of pts treated with antiplatelet drugs after percutaneous coronary intervention (PCI) with stent placement.

1663 PRELIMINARY DATA ON THROMBOPOIETIN RECEPTOR (CMPL) EXPRESSION IN DIFFERENT PLATELET DISORDERS
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Background. Interaction between thrombopoietin (TPO) and cMPL appears to be critical in the platelet production, promoting CD34+ differentiation, megakaryocytes (MK) proliferation and maturation via cytoplasmatic down-stream signalling. Another consequence of TPO-cMPL interaction is the regulation of TPO level. It is know that TPO is high in thrombocytopenia secondary to MK hypoplasia but TPO levels are low in immune thrombocytopenia (ITP) as well as in inherited macrothrombocytopenia and variable in essential thrombocythemia (ET). The aim of these study is to investigate cMPL expression in different cases of thrombocytopenia to better understand the therapeutic use of TPO mimetics. Patients and methods We enrolled 75 patients suffering from thrombocytopenia and divided them into acquired platelet disorder (21 pts) with an inherited macrothrombocytopenia (FAM) (MYH9 related disorder, Bemard Soulier classical, N41H or type2), 25 patients with a thrombocytopathy secondary to megacaryocyte hypoplasia (MK-Hyp) and 15 ET patients. We enrolled also 25 healthy subjects as control group. cMPL (82 kD, glycosylated) expression has been evaluated by Western-Blot on 2 x10⁷ platelets lysates using 1 mg/ml rabbit polyclonal anti-Human cMPL antibody. Antibody CD41-clone S2Z2 monoclonal antibody against platelet GpIib complex was used as control on the same membranes, after stripping anti-cMPL antibody. Expression of both cMPL and anti CD41 was quantitated by densitometry analysis of Western Blot. The results are given as mean± SEM of cMPL/CD41 expression ratio for each group of patients. Serum thrombopoietin levels have been evaluated by an ELISA assay, using a trade kit (Quantikine, R & D System). Results ITP (0.17±0.04), FAM (0.19 ± 0.06) and MK-Hyp (0.18±0.04) patients have higher cMPL expression than controls (0.04±0.01) and ET (0.0±0.01). There were no differences between controls and ET. TPO (pg/ml) in ITP (149±23) is higher (p=0.001) than controls (67±14), MK-Hyp have TPO levels (833±301) higher than controls (p=0.008) and ITP (p=0.02). All three form of thrombocytopenia have TPO level higher (p<0.05) than ET (respectively ITP 149±22, MK-HYP 833±301, FAM 98±15 pg/ml vs ET 55±5 pg/ml). No correlation between TPO and cMPL has been demonstrated in our groups. Conclusions: Our preliminary data demonstrate that TPO levels are higher in ITP, but not in ET. These results are in agreement with the fact that TPO levels are increased in ITP and in MK hypoplasia and are decreased in ET. It is possible that TPO level is regulated by cMPL expression. TPO regulates cMPL expression and cMPL modulates TPO action. Therefore, TPO may be a useful tool to study the role of cMPL in thrombopoiesis. We are currently analyzing the cMPL expression in other disorders of the platelet production.

1664 COMPLETE ITP REMISSION IN ADULTS FOLLOWING THE SPLENECTOMY IN LATVIA
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Background. Immune thrombocytopenic purpura (ITP) manifests as decrease in platelet (PLT) count of various degrees, which can even cause fatal bleeding. Glucocorticoids (GC) and intravenous immunoglobulin (IgG) are used as the first line of treatment; splenectomy is “the second stage”.

Aims. Determine the rate of complete remission following the splenectomy patients with ITP. Methods. The study includes 8 women and 5 men, the median age are 37 (IQR=18) years. The median duration of the disease prior to the surgery was 24 (IQR=80) months. Prior to the surgery 2 (15.4%) patients received only GC therapy, 5 patients (38.5%) received...
GC and immune suppressive (IS) therapy, in 5 cases (38.5%) GC and IgG was applied. One patient, for whom the duration of the disease prior to the surgery was 7 months, had received GC, IS and IgG therapy (7.2%). All patients received pneumococcal vaccination prior to the surgery. The median size of the spleen was 10.2 (IQR=2.1) cm detected by ultrasonography. In one US case and in one CT case accessory spleens were detected, which were removed during the surgery. 11 LS and 2 OS were done. Corticosteroid therapy (median value of PLT 58 x109/L (IQR=95) had increased in a statistically significant way in accordance with Wilcoxon signed-rank test (z=2.76; p=0.006) compared to the median value of PLT 185 x109/L (IQR=220), which was detected during the last follow-up on median 36 (IQR=55) months after the surgery. Complete remission (PLT count 250,000 mm3) was achieved in 7 cases (43.8%) and PLT levels increased in a statistically significant way in accordance with Wilcoxon signed-rank test (z=2.76; p=0.006) compared to the median value of PLT 185 x109/L (IQR=220), which was detected during the last follow-up on median 36 (IQR=55) months after the surgery. Conclusions. The splenectomy is safe and effective second stage procedure for patient with ITP in our study. Complete remission of ITP was observed in all cases.

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PLATELET GLYCOPROTEINS AND STICKY PLATELET SYNDROME

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Background/Aims. Sticky platelet syndrome (SPS) is a hereditary, autosomal dominant thrombophilia associated with an increased incidence of arterial and venous thrombosis. Light transmission aggregometry (LTA) is used to confirm a platelet hyperaggregation induced by very low concentration of platelet inducers - by adenosinediphosphate (ADP) and epinephrine (EP). Etiology of SPS is unknown but some studies suggest that abnormally high concentrations of P-selectin on the surface of platelets may play a role in the hyperfunction. Aim of the study was to verify if there are some abnormalities in the expression of platelet membrane GP receptors: P-selectin (CD62P), CD63 and CD51. The control group included 30 healthy individuals. All patients and controls agreed with participation in the study and signed an informed consent. Results: The results of flow cytometric analysis of SPS patients have shown that there is a significantly higher expression of P-selectin (CD62P), CD63 and CD51 compared to healthy controls (P-selectin: p<0.001; CD63: p<0.05; CD51: p<0.05 respectively). GP receptors are neoantigens expressed on the platelet surface only after platelet activation. Summary/Conclusions. On the basis of our measurements we can say that platelets in SPS patients are activated compared to the controls. We suggest that the increased expression of CD63 and CD51 may serve as predictors of thrombophilia in SPS patients.

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IDIOPATHIC THROMBOCYTOPENIC PURPURA IN ADULTS IN THE LAST 10 YEARS: SINGLE CENTRE EXPERIENCE

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Background. Adult idiopathic thrombocytopenic purpura (ITP) is a benign disease with low morbidity and mortality and frequent remissions that occur spontaneously or in response to first line treatment with steroids or splenectomy. Aims. The aim of this study is to analyze the clinical outcomes of 170 patients with ITP diagnosed and/or treated in our hospital in the period between 2000 and 2010. Methods. We retrospectively have analyzed the institution database for patients diagnosed and/or treated for ITP. All patients met the diagnostic criteria for ITP. Results. The median age at diagnosis was 47 years (range 14-83). 46% were males and 54% were females. Forty five (27%) patients had severe thrombocytopenia (Plt <30x109/L). Number of patients with severe thrombocytopenia (Plt <30x109/L) was 125/170 (75.5%), with moderate thrombocytopenia (Plt 30-50x109/L) was 14.1% and with mild (Plt 50-100x109/L) was 12.4%. Bleeding symptoms were more common in patients with severe thrombocytopenia. 98 (45.6%) patients belonged to 58 (33.9%) patients with moderate thrombocytopenia (Plt <30x109/L) and with mild (Plt 50-100x109/L) was 12.4%. Bleeding symptoms were more common in patients with severe thrombocytopenia. 108/125 (86.4%) patients, comparing to 45.8% of patients with moderate and 38% with mild thrombocytopenia (p<0.001). Bone marrow examination was performed in 76% of patients. Anti-platelet antibodies were positive in 8/35 (23%) of patients. Direct antiglobulin test was positive in 7/155 (5.2%) patients and 2.9% patients had hemolytic anemia. Median follow up of all patients was 13 months. Ninety five patients had follow up longer than 12 months, with 12 months (range: 12-108). Corticosteroids were initial treatment for 95% patients with severe thrombocytopenia was 12/170 (7.1%), with moderate was 12/71 (17%), with mild 33/194 (12.4%) and with platelet count >100x109/L was 66.4%. At the last follow up, number of patients with severe thrombocytopenia was 12/170 (7.1%), with moderate was 12/71 (17%), with mild 33/194 (12.4%) and with platelet count >100x109/L was 66.4%. At the last follow up, number of patients with severe thrombocytopenia was 12/170 (7.1%), with moderate was 12/71 (17%), with mild 33/194 (12.4%) and with platelet count >100x109/L was 66.4%. Conclusions. The results from our study are similar with previously reported in view of median age, sex distribution, hemorrhagic symptoms, response rate etc. Our results approved that most adults with ITP have a good outcome with low morbidity and mortality. But almost 30% of patients had refractory ITP; which represent significant medical problem due to a need of new treatment.

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MEAN PLATELET VOLUME DECREASED AFTER IMMUNOAPHERESIS OR CASCADE PLASMA FILTRATION THERAPY IN FAMILIAL HYPERCHOLESTEROLEMIA

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Introduction: Platelet size, measured as mean platelet volume (MPV), is associated with platelet reactivity. If MPV would drop after LDL-erasing therapy, decreased MPV could be one of the simple markers of successful therapy (decreased atherosclerosis activity). Methods and patients: MPV was investigated in patients with severe familial hypercholesterolemia (FH) long-term treated (8-12 years) by LDL-apheresis (immunoapheresis) or cascade filtration. MPV was obtained by centrifugation. Adsorbers Lipopak 400® were used for immunoaapheresis and filters Evalux 4A® were used for cascade filtration. 95 pair samples were measured (before and after the procedures) - 8 times during 4 years in 12 patients. Results: Mean MPV before the procedures was 10.891 fl (range 9.04 - 11.11) - this difference is significant (p = 0.036). MPV did not correlate with age, sex, platelet count, duration of therapy. Discussion: Our study gives a good evidence of MCV drop after the described therapy; we did not find any other data about MCV changes in FH with extracorporeal elimination therapy in the literature. MPV is easily available and is often disregarded, but sometimes may indicate the need for a careful assessment in patients with FH. The association of the MPV with the risk of coronary and cerebrovascular diseases and atherosclerosis activity was reported. Conclusions. MPV could be one of the markers of therapeutic efficacy in patients with FH treated by extracorporeal elimination. MCV examination is a simple, inexpensiy and easily accessible method.

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1668

STUDY OF T-CELL IMMUNOGLOBULIN- AND MUCIN-DOMAIN-CONTAINING MOLECULE 3 POLYMORPHISMS IN PEDIATRIC EGYPTIAN PATIENTS WITH IDIOPATHIC THROMBOCYTOPENIC PURPURA

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Background. Idiopathic thrombocytopenic purpura (ITP) is an acquired autoimmune disease characterized by the production of autoantibodies that mediate platelet destruction. Dysfunctional T cell-mediated immunity plays an important role in the pathophysiology of ITP. In humans,
the T-cell immunoglobulin and mucin-domain (TIM) gene family, located on chromosome 5q35.2, consists of TIM-1, TIM-3, and TIM-4 genes, which encode cell-surface glycoproteins with similar structures. TIM-3 was reported as a central regulator of T-cell responses. Recently it was reported that TIM-3 mRNA expression in peripheral blood mononuclear cells was significantly lower in ITP patients than in healthy controls which indicated that TIM-3 may play an important role in the pathogenesis of ITP. One single study by Du et al (Human Immunology, 2009, 70:398-402) investigated the role of TIM-3 polymorphism in ITP in North China. Nonetheless, pharmacogenetic studies show marked ethnic variations. Also this study did not address the impact of the polymorphisms on severity and clinical course of the disease. Aims: (1) To study TIM-3 gene polymorphism as a risk factor for ITP in a cohort of Egyptian children. (2) To verify if any of the polymorphisms would have an impact on the severity or the clinical course of the disease. Methods: Under informed consent 100 patients (46 acute, 50 chronic and 4 persistent ITP cases) and 210 controls were tested for TIM-3 G1516T, T574G, and G4259T polymorphisms using PCR-RFLP. Allele frequencies were calculated for patients and controls. Patients were treated according to standard protocols. The impact of TIM-3 gene polymorphisms on severity and clinical outcome was studied. Results: No significant difference in the distribution of the wild, heterozygous and homozygous genotypes was encountered between cases and controls. Only TIM-3 T574G showed a significant difference (p=0.08) where GG, TG and TT constituted 43.88%, 50.12% and 5.1% in cases as compared to 54.28%, 38.1% and 7.62% in the controls. T and G allele frequencies were 30.6% and 69.4% in cases as compared to 26.67% and 73.33% in the control. Neither was there significant association between any of the polymorphisms on one hand and the severity of disease or the clinical outcome on the other hand. However near significant association (p=0.08) was encountered between 4259TT and severity as represented by bleeding at presentation (63.1% vs. 38.7%). On the other hand 574GG showed near significant association with better quality of response (71.42% in responders vs. 42.1% in non-responders, p=0.07). Similarly, 4259TT showed near significant association with better quality of response (57.8% in responders vs. 5.1% in non-responders, p=0.09). Conclusions: This study confirms the previous report that TIM-3 gene polymorphism might not play an important role as a genetic risk factor in the pathophysiology of ITP. Neither it has had a major impact on the severity or clinical course of the disease. Nevertheless studying a larger number of cases may further prove or disprove this notion.

1669 CHILDHOOD IMMUNE THROMBOCYTOPENIC PURPURA: A REGIONAL STUDY OF 140 CASES

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Introduction. Childhood immune thrombocytopenic purpura (ITP) is a rare disease. Physician management of childhood ITP is diverse. We report the clinical, therapeutic and outcome of childhood ITP in our hospital. Patients and Methods: We retrospectively analyzed cases of childhood ITP diagnosed in pediatrics and hematology departments of hospital Hedi Chaker Sfax, between January 1995 and December 2009. We analyzed the epidemiological, clinical, biological and evolution. Treatment response was defined as follows: complete response (CR), a platelet count ≥100000/mm3 persisting for at least 2 months, partial response (PR): a platelet count between 50-100000/mm3 and no response (NR): a platelet count <50000/mm3. Results: During the study period, 140 cases of ITP were collected. The mean age at diagnosis was 6 years (3 months-15 years). The vast majority of children had mild bleeding symptoms: purpura and petechiae (100%), epistaxis (55%), gum bleeding (26%). Intracranial hemorrhage occurred in one child. Eighty four (60%) children were treated with corticosteroids alone and seventeen children (12%) with intravenous immunoglobulins (IVIG). One hundred forty two (90%) patients achieved good response (CR+PR) to corticosteroids. A chronic evolution was noted in 30 cases (21%). Conclusions: The data from our series revealed that clinical characteristics and outcome of childhood ITP are similar to those series reported in literature. Corticosteroids remain the first-choice treatment for childhood ITP resulting in a response in 90%.

1670 CHRONIC IMMUN THROMBOPOEITIC PURPURA: RETROSPECTIVE ANALYSIS OF 30 CHILDREN

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Childhood immune thrombocytopenic purpura (ITP) is an autoimmune acquired disorder. Approximately 20% to 25% of children manifest chronic ITP. We analyzed clinical characteristics and management of children with chronic ITP. Patients and methods: We retrospectively analyzed the clinical, therapeutic and evolution of children manifest chronic ITP diagnosed in pediatrics and hematology department of hospital Hedi Chaker Sfax, from January 1995 to December 2008. Treatment response was defined as follows: complete response (CR), a platelet count>100000/mm3 persisting for at least 2 months, partial response (PR): a platelet count between 50-100000/mm3 and no response (NR): a platelet count <50000/mm3. Results: During the period of study, 140 cases of ITP were collected. 120 cases were analyzed in the department of haematology and paediatric. Twenty three children (21%) (22 girls and 8 boys) manifest chronic ITP at 6 months after the initial diagnosis of ITP. The mean age was 9 years. The distribution of cases according to age showed a maximum at 10-12 years. Twenty one children were treated in first line: with corticosteroid for 19 patients and intravenous immunoglobulin for 2 patients. Fourteen children, who were nonresponsive to first line treatment, 14 children were splenectomized and 2 children treated with immunosuppressive agents (vincristine, azathioprine). The follow up period after splenectomy ranged between 2 to 15 years. A complete remission (CR) was achieved in 78% cases without any relapse. There were no infections after splenectomy in any children.

1671 PLATELETS ASSOCIATED ANTIBODIES PRE AND POST SPLENECTOMY IN HEPATITIS C PATIENTS WITH THROMBOCYTOPENIA

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Background: The presence of platelet antibodies inducing thrombocytopenia in patients with HCV infection is a matter of great debate. Subjects and methods: The present study was conducted on 23 subjects categorized as follows: Group 1: included 23 cases diagnosed as chronic hepatitis C before splenectomy. Group 2: included the same 23 cases after splenectomy. For all subjects included in this study platelets counts was evaluated as well as platelets associated antibodies (IgM, IgG, IgA). Results: All patients were thrombocytopenic before undergone splenectomy. Post splenectomy platelet counts (51.8±16.7). After splenectomy all patients were of normal platelet counts (174.2±55.8). The mean ±SD of platelets associated immunoglobulin were (64.2±9.6) for total lgs, (55.6±8.1) for IgM, (3.8±2.1) for IgG, (6.7±4.7)presplenectomy versus post-splenectomy for total lgs (15.4±19.3), for IgG (5.4±1.8), for IgM (1.9±0.6), for IgA (2.1±0.9) and the differences was statistically significant (P<0.001). The correlation between hospital and platelet counts and total PAIgs level in patients with chronic HCV infection pre and post splenectomy revealed that there is significant correlation between platelets counts and total Ig (r =0.804, P =0.000), (r-0.907 P =0.000), (r -0.467, P =0.002), (r -0.519, P =0.000). Conclusions: autoimmune mechanism plays an important role in the HCV associated thrombocytopenia and spleen is a major source of PAIgs.

1672 SERIOUS THROMBOCYTOPAENIA AS HEMATOLOGICAL MANIFESTATION OF NOONAN SYNDROME

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Serious thrombocytopenia has been reported in Noonan Syndrome although thrombocytopenia was not recognized mainly as first clinical manifestation. Abnormal platelet count or function could occur in this disorder. Boy, first child of nonconsanguineous parents, polyhydramnios diagnosed at 26 weeks of gestation. Birth weight was 2560 g (F5) and length was 45 cm (P<5), admitted for bleeding diathesis in the first day of life. On physical examination, petechial and purpuric rash, dysmorphic facies: low-set ears, high forehead, hypertelorism, and
bilateral cryptorchidism, syndactyly of four and five left foot fingers. There was no evidence of hepatosplenomegaly. Cardiologic investigations revealed an aortic valvar dysplasia with minimal insufficiency and atrial septal defect. An initial complete blood count showed a platelet count of 5,000/ul. Evaluation of his neonatal thrombocytopenia included a platelet immune workup and a congenital infection workup; neither yielded positive results. Familial thrombocytopenia and the thrombocytopenia absent radius syndrome were ruled out. The patient had given several platelet transfusions and immune globulin over the first weeks of life because of repeated low platelet counts. The PT and PTT were within normal limits. Bone marrow aspiration was normal. Genetic analysis revealed the presence of a constitutive 218 C→ G mutation in exon 6 of the F8TN11 gene that was associated with Noonan Syndrome. We present this case because Noonan Syndrome was rarely described as cause of serious inherited thrombocytopenia. This mutation was identified in several patients with juvenile myelomonocytic leukemia, hematological follow up is important in these patients.

**1673**
**CARBAMAZEPINE-INDUCED IMMUNE THROMBOCYTOPENIA AND RELATED WITH TO PHENYTOIN**

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*Background.* Phenytoin and carbamazepine are rarely associated with serious hematological side effects. Carbamazepine (CBZ) is an effective anticonvulsant for children with partially and secondarily generalized seizures. It is considered safe and relatively less toxic than other anticonvulsants. The hematological toxicity of CBZ is well known and thrombocytopenia is a rare manifestation of CBZ-induced myelotoxicity. Thrombocytopenia is a rare manifestation of hematological toxicity of CBZ, and results from myelosuppression which occurs within four weeks of initiating the treatment. Case report: A 10-year-old boy was admitted with generalized, non-itchy erythematous body rash. There was no associated fever, conjunctival hyperemia, oral ulcers or other systemic manifestations. Three months before the presentation, he had been administered a general seizure and was started on 5mg/kg/day of phenytoin in two divided doses. He developed severe urticarial lesions. Di-phenylhydantoin was discontinued and the patient was switched over to CBZ. There was no previous history of bleeding diathesis or drug allergy. Examination revealed extensive purpura and petechiae mainly over the limbs and a few scattered lesions over the trunk. There was ecchymoses, wet bleeds and epistaxis. The child had no pallor, bone-tenderness or lymphadenopathy. Abdominal examination revealed no hepatosplenomegaly and splenomegaly. Investigations revealed hemoglobin 12g/dl, total leukocytes count 8,000/mm3 with 69% neutrophils, 32% lymphocytes, 2% eosinophils and 4% monocytes. Platelet count was 3,000/mm3. Anti-nuclear antibody was negative. Bone-marrow aspiration showed decreased megakaryocytes with no abnormal cells. CBZ was discontinued and mega-dose steroid treatment was started. There was a rapid recovery with platelet count rising to 15,000/mm3 on the second day and further to 518,000/mm3 on the fifth day of starting prednisolon. The patient has remained seizure-free and thrombocytopenia has not recurred did not occur during a follow-up of one month. Results: Thrombocytopenia is a rare manifestation of hematological toxicity of CBZ, and results from myelosuppression which occurs within four weeks of initiating the treatment. In our patient, ten days after beginning CBZ treatment, thrombocytopenia developed. Thrombocytopenia usually resolves once CBZ is discontinued. We used mega-dose steroid treatment for serious thrombocytopenia and epistaxis. Bone marrow examination is usually required to differentiate it from primary bone marrow disorder. Summary / Conclusions: Hematological monitoring is recommended the patient who developed phenytoin toxicity since it does not identify the patients at risk of serious blood dyscrasias. Physicians prescribing CBZ should be aware of phenytoin toxicity; we advise another anticonvulsant therapy that developed side effects of phenytoin.

**1674**
**BENEFICIAL EFFECT OF EARLY ADMINISTRATION OF RITUXIMAB TREATMENT FOR ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA**

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*Background.* Plasma exchange (PE) is first-line therapy for patients with acquired thrombotic thrombocytopenic purpura (TTP) because of the clearance of circulating ADAMTS13 inhibitors. However, there is a subset of patients who do not initially respond to PE treatment or subsequently deteriorate while on PE treatment around day 7-14. There are limited reports that describe detailed clinical and serial laboratory findings during this challenging process. The exact pathophysiology of this deterioration, we generally refer to as inhibitor boosting, remains still uncertain, furthermore, the appropriate management or prophylaxis is also unclear. Patients for refractory TTP are at greater risk of complications and, therefore, early administration of Rituximab might be a considerable option, which could reduce plasma requirement and avoid complications. Here we describe four patients with severe acquired TTP who were successfully treated with Rituximab. Aims: The purpose of presenting our cases is to highlight how early initiation of Rituximab could lead to a positive outcome, especially in a severely ill patient. We also present detailed laboratory analysis, including frequent ADAMTS13 measurements, during a phase of the ‘inhibitor boosting’. Methods: We reviewed all the patients with acquired TTP treated with Rituximab at Tenri Hospital, 4 cases were identified from 2006 to 2010. Clinical and laboratory findings, including ADAMTS13 activity and inhibitors, were recorded and analyzed.

**Results.** All 4 patients (median age 67, range 40-78) showed severely decreased ADAMTS13 activity (<1.0%) with detectable inhibitor level (median 3.4 Bethesda U/ml, range 1.72-6.80). 3 patients presented during their first TTP episode while one patient had relapse of TTP after 10 months remission. We immediately treated all patients with PE at diagnosis with or acquired TTP. However, in two cases of them, we subsequently administered Rituximab therapy because of deterioration while on PE treatment. At this time point (one patient at day 7, another at day 14), ADAMTS13 activity or inhibitor testing revealed considerable abnormal results. In another two high-risk cases, which were a recurrent case and a severe acute case of deep coma as the first episode, we administered upfront Rituximab therapy after only a few PE treatments. After the addition of Rituximab (375mg/m2 each week for 4 week), all of the 4 patients achieved complete clinical and hematological remissions with rapid recovery of ADAMTS13 activity and resolution of detectable inhibitor. All patients required no additional plasma exchange after completion of rituximab and remained in sustained remissions with median follow-up of 30 months (range, 3-60 months).

Discussion: In refractory and recurrent cases, administration of Rituximab appears to be a reasonable therapeutic option. In our limited experiences, early up-front addition of Rituximab, before deterioration, may be also beneficial in avoiding exacerbation, decreasing the need for plasma exchange and sustaining long-terms remission. Prospective studies are needed to define the true efficacy and safety of up-front Rituximab therapy and timing of its initiation.

**1675**
**RESPONSE TO THROMBOPOIETIN ANALOGUES (ROMIPLOSTIM) AS SECOND LINE TREATMENT IN SIX PATIENTS WITH IMMUNE THROMBOCYTOPENIC PURPURA REFRACTORY TO CORTICOSTEROIDS**

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Aims. Evaluation of the efficacy and safety of rituximab as salvage treatment in children with the diagnosis of chronic refractory ITP. A prospective unicenter study regarding the primary outcome data of rituximab therapy in children with chronic ITP in the pediatric setting remains to be established. Previous results of rituximab in adults are promising, treatment doses, and follow-up time from initial diagnosis till rituximab treatment 1.28 years. Previous treatment included for 5 children (71.4%) combined treatment with corticosteroids and IVIG, for 1 child (14.2%) corticosteroids, IVIG and anti D globulin, and for 1 (14.2%) corticosteroids, IVIG, anti D globulin and splenectomy. Rituximab was administered intravenously at a dose of 575 mg/m² weekly, for a total of four infusions in 4 patients (71.4%) and 1 single dose in 2 children. CR was recorded in 5/7 children (71.4%) with median PLT 149 x 10⁹/L and median follow up time 25.3 months. PR and NR was recorded in 1 child and 1 child respectively. Immunophenotype analysis revealed no circulating B CD 19+ (<1%) in six children and no significant B cell depletion (>4%) in one child (poor responder). Side effects were mild and transient and were recorded in only 1 of 7 patients (14.2%) with fever and cough which was successfully treated with antimicrobial agents. Conclusions: Rituximab therapy is beneficial for some children with severe chronic ITP who are refractory to standard agents. The toxicity profile of rituximab is acceptable in most patients. Further studies are needed to determine the optimal treatment schedule, the real rates of efficacy before and after splenectomy, the long-term side effects, and the drug’s mechanism of action.

Efficacy and Safety of Rituximab in Children with Chronic Immune Thrombocytopenia: A Single Institution Experience

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Background. Recent recommendations concerning primary immune thrombocytopenia (ITP) in childhood defines ITP as an acquired immune mediated disorder characterized by isolated thrombocytopenia, defined as a peripheral blood platelet count less than 100 x 10⁹/L, and the absence of any obvious initiating and/or underlying cause of the thrombocytopenia. Furthermore, a recently approved and promising treatment option in chronic ITP in childhood is reported and concerns rituximab. Rituximab is a chimeric monoclonal antibody against the protein CD20, which is primarily found on the surface of B cells, inducing B cell destruction and autoantibodies depletion. On the other hand, although previous results of rituximab in adults are promising, treatment doses, efficacy and safety in the pediatric setting remains to be established.

Aims. Evaluation of the efficacy and safety of rituximab as salvage treatment in children diagnosed with chronic ITP. Materials and Methods. A prospective unicenter study regarding the primary outcome data of rituximab therapy in children with the diagnosis of chronic refractory ITP was performed. The treatment response was evaluated after the every course of rituximab treatment. Duration of response was considered from the day of the initial infusion to the first time of relapse or to time of analysis. Complete response (CR) was defined as PLT count >100 x 10⁹/L, partial response (PR) was as any PLT counts between 30 and 100 x 10⁹/L and no response (NR) below 30 x 10⁹/L. Relapse was also defined as a decrease of the platelet count to below 30 x 10⁹/L after CR or PR. The patients received no other treatment in addition to rituximab. Results: Eight children out of 13 with chronic ITP (53.85%) received rituximab - 85.7% females- with mean age 9.5±1.6 years, mean PLT count 19x10⁹/L and the average number of platelets was 34,000 with hemorrhagic symptoms. Hemorrhagic symptoms disappeared in all cases except one, a female patient who did not respond despite escalation to the maximum dose of 10 μg/kg/week. Since her diagnosis was later changed to megakaryocytic purpura (47, XX, +8), she was excluded from the response analysis. Four of the five remaining patients obtained a partial response (PC > 30 x10⁹/L) within a week of treatment initiation (mean dose 2.5 μg/kg/week), and all of them achieved complete response (PC > 100 x10⁹/L) after a mean of 31.4 days (mean dose 2.5 μg/kg/table). All patients tolerated the treatment without clinically relevant side effects, except mild headache or bone pain, and were able to return to work, considerably improving their quality of life. Conclusions: Thrombopoietin analogues are a treatment option for patients with chronic immune thrombocytopenia to corticosteroid therapy, in whom other therapies such as splenectomy are contraindicated. In these patients, the administration of low doses of romiplostim, with the aim of restoring safe platelet counts, provides satisfactory responses from a clinical point of view, with safe platelet counts, decreased hemorrhagic symptoms and improved quality of life, with little or no adverse effects. Thus, thrombopoietin analogues are safe and effective as first choice for second line treatment of severe chronic ITP.

Efficacy of Rituximab in the Treatment of Idiopathic Thrombocytopenic Purpura

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Background. The objective of this study was to report the results of splenectomy in the treatment of idiopathic thrombocytopenic purpura (ITP) in adults. Materials and methods: This is a retrospective study between January 1994 and December 2009 at the hematology department of the University Hospital of Sidi Bel Abbès. All patients had ITP and received initial treatment with corticosteroids. In case of steroid resistance or dependence, splenectomy was performed. Results. Of the 60 selected ITP, 18 splenectomy were performed (30%) among 15 women and 3 men with a mean age of 33 years. Before splenectomy, the average number of platelets was 34,000 with hemorrhagic manifestations in three quarter of cases. Two weeks after surgery, there was a complete remission (CR) in 13 cases (72%), while partial response (PR) was noted in 3 cases (17%) and failure in 2 (11%). Of the 13 patients in CR, one relapsed after a period of 12 months. The 5 patients with PR have relapsed after a median time of 24 months. Finally, 12 patients (66%) are still in CR with a minimum follow-up of 18 months. Two factors of response to splenectomy are noted: the age of the patient below 50 years and the initial response to corticosteroids. Conclusions. Splenectomy is an important therapeutic modality of ITP with immediate effect, but especially in the long term of at least 60% responses.

Novel Platelet Indices in Patients with Homozygous or Heterozygous Sickle Cell Anemia Compared to Iron Deficiency Anemia

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Background. Modern hematological analyzers have simplified registration of platelet indices, such as mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and immature platelet fraction (IPF), which allows further study of these indices in disorders of hemopoiesis. Aims. The aim of this study was to compare the aforementioned platelet indices in patients with iron deficiency anemia and sickle cell anemia. Methods. We retrospectively studied patients with heterozygous sickle cell anemia (n=15, 7 female and 6 male), homozygous sickle cell anemia (n=92, 54 female and 38 male) and iron deficiency anemia (n=42, 26 female and 16 male). Hematological parameters were assessed by SYMEX XE2100 hematological analyzer. SPSS Statistics for Windows, version 17.0 was used for statistical analysis. The significance level was defined as p<0.05. Results. The three patient groups were comparable as for age and sex. Mean PCT value was 0.259±0.118% for heterozygotes, 0.4147±0.1211% for homozygotes, and 0.2947±0.12174% for iron deficient patients. Mean PCT value differed...
statistically significantly between homozygous and heterozygous sickle cell anemia patients (p<0.001), as well as between homozygous sickle cell anemia and iron deficiency anemia patients (p<0.001). Statistically significant positive correlations between IPF and MPV, as well as MPV and PDW were noted in homozygous sickle cell anemia patients (r=0.687, p<0.001, and r=0.651, respectively) and iron deficiency anemia patients (r=0.756, p<0.001, and r=0.768, p<0.001, respectively). Correlations Plateletcrit value was significantly higher in patients with homozygous sickle cell anemia, compared with homozygous and patients with iron deficiency anemia, while no significant difference was observed between heterozygous and iron deficient patients. As for correlations among platelet indices, IPF was positively correlated with platelet indices MPV and PDW in homozygous patients with sickle cell anemia and in patients with iron deficiency anemia.

A STUDY ON PLATELET INDICES IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Large platelets are more active and thus more thrombogenic than smaller ones, as they contain more dense granules, they produce greater amounts of vasoactive and prothrombotic factors and present an increased expression of adhesion molecules. Mean platelet volume (MPV) and platelet distribution width (PDW) are indicators of platelet function and activation and MPV has been reported to be influenced inversely by inflammation. Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder with various hematological manifestations. Altered platelet indices have been found in patients with RA and it remains intriguing to investigate the correlation of coagulation and inflammation in RA. Aims: The present study aims to examine the changes in platelet indices (platelet count, MPV and PDW) in patients with RA, and their interaction with biomarkers reflecting the inflammatory response (C-reactive protein (CRP), erythrocyte sedimentation rate (ESR)). Methods: The study group consisted of 25 RA patients (males/females: 5/20, mean age: 47 years) at various disease phases and 30 age and sex-matched healthy controls. Platelet indices were measured with hematological analyser XE-5000 SYMEX (ROCHE) with flow cytometry method within 1h of sampling in order to avoid the time-dependent swelling by EDTA. CRP was determined with the use of Immage 300 nephelometer (Beckman Coulter) and ESR with Ves-matic 20 (Diseo) analyser. Student parametric test (t test) and Pearson’s correlation were used for the statistical analysis. P<0.05 was considered statistically significant. Results: The laboratory parameters are reported in Table 1. Platelet count, MPV and PDW have been found to be significantly higher (p<0.05) in RA. MPV values above the reference range were detected in the majority of patients tested (76%, 19/25). Platelet count was positively correlated with CRP and ESR in RA patients (p<0.05). The other platelet indices presented a positive but not statistically significant correlation with the examined inflammatory markers.

Summary/Conclusions: In RA patients alterations in platelet indices, indicative of platelet activation, were observed. Only thrombocyte count was correlated with the inflammatory markers tested. The association of platelet routine laboratory parameters with inflammation in patients with autoimmune inflammatory disorders and their clinical utility for the rapid assessment of the disease activity should be further investigated.

SECONDARY POLYCYTHEMIA DURING THE COURSE OF IMMUNE THROMBOCYTOPENIC PURPURA (ITP) TREATMENT WITH ROMIPLOSTIM

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Background: Involvement of thrombopoietin (TPO) on erythropoiesis has been described in the literature (1). Then we can ask whether the polycythemia can occur as a side effect of TPO analogue, as Romiplostim. We report 2 cases of patients treated with Romiplostim and increased hemoglobin level. Case 1: an asymptomatic thrombocytopenia (5.109/L) found before a surgery lead to a diagnosis of ITP in June 2009, in a 40 years old man. Initial evolution was unfavorable with corticosteroids and intravenous immunoglobulin (IVIG) with the occurrence of relapses requiring repeated courses of IV Ig. Treatment with Danatroil and Rituximab were not efficient. Romiplostim was introduced in December 2009 with a good response obtained at a dose of 3 mg/kg. In correlation with therapy initiation, appeared polycythemia with a hemoglobin level that reached 180 g/L in few days (13 g/L previously) without organic cause. Dose reduction was performed and provided a decrease on hemoglobin level. Case 2: a 74 years old man with the history of refractory ITP (including splenectomy) diagnosed in 1995, received in November 2009 a treatment with Romiplostim. Polycythemia appeared rapidly with a rise of hemoglobin level to 170g/L (versus 145 g/L before). A stable level of platelets was difficult to obtain requiring multiple variations of doses, which allows us to identify a positive relationship between the dose of Romiplostim and hemoglobin level. Conclusion: An increase in hemoglobin level was found in these 2 patients, and this soon after starting treatment with Romiplostim. This fact can suggest imputability of the drug in polycythemia, excluding other causes. Larger series will be necessary however to confirm this hypothesis.

References

PLASMA EXCHANGE IN THE TREATMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA - ONE CENTRE EXPERIENCE

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Background. Plasma exchange (PE) has changed significantly the prognosis of thrombotic thrombocytopenic purpura (TTP), so that remission occurs in more than 80% of patients, as is documented by the literature and our own 30-year experience. In spite of this several details remain to be cleared. Aims. We performed a retrospective analysis of therapy results of PE in TTP during the last 10 years when therapy at our workplace had been standardized. Methods: In the years 2000 - 2010 all patients sent for the therapy to our regional centre were evaluated. (The centre serves for a region of about 1.5 million inhabitants). All 40 adult patients (25 females, 15 men, mean age 43, range 20-79 years) had 66 episodes (initial treatment or relapses). PE was performed using Cobe Spectra separator (Caridpan, Lakewood, Colorado, USA), software 7.0. Every day one plasma volume was replaced with fresh frozen plasma. ACD-A was used as anticoagulation fluid (Baxter, Munich, Germany). After the rise of thrombocytes above 150x109/L, PE was performed in gradually prolonged intervals. Results: In all 40 patients where TTP was confirmed (characteristic pentad of symptoms or at least unexplained thrombocytopenia + microangiopathic hemolytic anemia), PE was started within 4 hours. In initial therapy or in relapses 57 PE in total were performed (mean 14.5, range 3-59; median 9). Inherited TTP with
ADAMTS13 deficiency was confirmed in 3 patients, 12 cases were idiopathic, 7 patients had organ malignancies, 9 patients were after stem cell transplantation, myelodysplastic syndrome or myeloproliferative diseases had 3 patients, autoimmune diseases 3 patients, pregnancy 3 patients. Relapses occurred in 9 (22%) patients (mean number of relapses was 3.8, range 2 - 7, median 4). Within 30 days after initiation of PE 8 patients showed thromboembolism in all patients with malignancies and after stem cell transplantation and in 3 patients with idiopathic TTP. All patients received corticosteroids. Splenectomy was performed in 4 patients with relapse. 10.8% of side-effects were mostly allergic, none was clinically serious. Conclusions: Even with improved prognosis TTP remains a serious disease. All patients suspected of TTP should receive urgent PE which in our cohort was successful in 80% of cases but nev er in organ malignancies after transplantation. PE is the key therapy in TTP - its early performance is a life-saving measure. PE is a safe method in hands of a skilled staff.

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### 1682

**IMPLEMENTATION OF THE NEW HEP SCALE FOR THE EVALUATION OF HEPARIN-INDUCED THROMBOCYTOPENIA**

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Alterations of hemostasis and blood counts are common in patients admitted to ICU. Clinical, laboratory and therapeutic factors.

1. Analyze the incidence of thrombocytopenia in patients admitted to ICU: clinical, laboratory and therapeutic factors.

2. Reclassify the patients undergoing cardiac surgery according to different scales that assess the likelihood of heparin-induced thrombocytopenia (HIT). Methods and patients.

A retrospective study by reviewing medical records of patients with thrombocytopenia (platelets <15x10^9/L) admitted to four ICUs of our hospital during a 40 days period, classified as mild (100-50x10^9/L), moderate (50-20x10^9/L) and severe (<20x10^9/L). Evaluation of patients undergoing cardiovascular surgery with cardiopulmonary bypass, classifying them according to the new scale presented as pre-test model for Cuker et al. and the 4 T’s model (Journal of Thrombosis and Haemostasis 2010; 8: 2642-2650): RESULTS: 237 patients admitted during this period, 50 showed thrombocytopenia (21%) Cumulative incidence: 19.8% in 40 days, excluding those who previously had his income. Men 56%, Women 44%, mean age 70.7 (extremes 19-88). Depending on different UCI: Coronary 9 patients, mean age 67.5, cardiovascular 25 patients, mean age 70.0; Polyvalent 11 patients, mean age 65.8; Traumatology 6 patients, the mean age: 75.6. Reason for admission: 32% valve replacement, 26% infection, 14% ischemic heart disease 14%, respiratory 8% hepatoctomy 8%, coronary artery bypass surgery 8%, ruptured aneurism 4%, bleeding 4%, decreased consciousness 4%, other 6% Mild thrombocytopenia: 94%, moderate: 0%, severe: 6%. Nadir figure rating: 90x10^9/L. Average time duration of thrombocytopenia: 42 days, median: 2.5 days. Etiology: Image 1. Distribution due to drug: abciximab 8%, tirofiban 2%. Incidence bleeding: 30%, with major bleeding (10%) located in: mediastinal 2%, hematoma in back with 2% drop in hematocrit, HDA 2%, brain 2% pulmonary and 2%, the rest were minor bleeds. Thrombotic phenomenon not observed.

**Transfusion:** CH 54%, CP 22%. Incidence of coagulopathy: 30%. Regarding the administered treatment: plasma 12%, vit K 8% FVIIIr and other measures 2%. Vitamin K and plasma 2%. The attributable mortality: 10 patients (20%). Of 78 patients undergoing cardiac surgery 25 showed thrombocytopenia (32%) and all of them had a low probability of HIT in both the 4 T’s scale as in the HEP scale, presenting both a score of <= 3 and <= - 3, respectively. Conclusions: 1) Surgery bypass is the most common cause of thrombocytopenia, because of our center location and previous causes. 2) In most cases, thrombocytopenia is mild and transient, short-lived, and have not needed treatment. 3) The classification of patients with the proposed scales.

### 1683

**SERENOA REPENS AS A CAUSE OF DRUG-INDUCED THROMBOCYTOPENIA. APROPOS OF TWO CASES**

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**Introduction:** In the differential diagnosis of isolated thrombocytopenia should be considered drug induced. There are extensive listings of agents with causal relationship to the thrombocytopenia is likely although few are final. The diagnosis can only be done by the recovery of thrombocytepenia after discontinuation of the drug and confirmed his comeback to reintroduce it. Serenova repens is a plant used for benign prostatic hyperplasia in herbal products and pharmaceutical specialties. CASE 1: 65 year old male visited the emergency department for hematuria, and spontaneous hematoma in the right hemithorax. He reported influenza vaccination for 15 days and a new treatment in herbal product (Sabal extract) He had platelets/mm3 14000. Studies found no cause of thrombocytopenia. It began as emergency therapy steroids (1 mg / kg / day) and suspended Sabal extract. The patient’s platelet count normalized in 7 days without subsequent relapse (follow-up two years). CASE 2: A 45-year-old, came to our Unit with cutaneous purpura mucosa detected platelets/mm3 3000. He complained of recent gastroenteritis and a contact with a young girl of his family suspected of having monoclonus. He reported no chronic treatment. He started steroids (1 mg / kg / day), reaching normal levels of platelets in 15 days. Serology was found positive for C Hepatitis so he received treatment with interferon-ribavirin. Three years later the patient presented again with thrombocytopenia (5000 platelets / mm3) and petechiae. Had no response to interferon-ribavirin but was stable in the liver. Receiving steroid treatment the platelet count normalized within a week. After insisting on the history the patient reported that days before the first episode had begun treatment with Neo-urgenin® (contains Serenova repens) He discontinued the treatment on his own soon after starting steroids. Two weeks before the relapse he restarted the treatment because he had prostatic symptoms. Discussion: The present cases suggest that thrombocytopenia in both patientes was induced by Serenova repens. The drug was started a few days before the clinical detection of bleeding and thrombocytopenia. In both patientes and second in both events) thrombocytopenia disappeared quickly stop taking the drug. Although in the two patientes steroids were empirically used that fact does not invalidate the diagnosis. The fact that in the second case to restart treatment with Serenova repens caused a new episode of thrombocytopenia appears to confirm the involvement of this substance in the genesis of thrombocytopenia.

### 1684

**ACQUIRED INHIBITOR OF FACTOR VIII ASSOCIATED WITH PROSTATE CANCER - A CASE REPORT**

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Background. Acquired haemophilia (AH) is rare disease that occurs at a rate of approximately 1.0 per million each year, although it is likely that not all affected patients are reported. It is a severe bleeding diathesis that affects both males and females, caused by sudden appearing autoantibodies that interfere with coagulation factor VIII (FVIII) activity. In the approximately half of patients with inhibitors, no concomitant disease can be found, and in the conditions most commonly associated with factor VIII inhibitors include connective tissue disease, inflammatory bowel disease, some dermatologic disorders and malignancy (lymphoproliferative disorders and solid tumours, e.g. neoplasms of the colon, pancreatic, breast, prostate, testis, lung). Thrombotic manifestations are often severe and may occur spontaneously or after minor trauma. In contrast to patients with congenital factor VIII deficiency those with acquired haemophilia principally experience soft tissue bleeding. Over-
all mortality associated with AH has been reported at between 8% to 42%. The aim: The aim of the presentation is a case of inquired haemophilia in the course of prostate cancer. Case report. A 71-year-old man presented with sacrosphincter, left femoral and brachial muscle non traumatic haematoma and spontaneous hematuria, but otherwise felt well. A coagulation screen was as follows: platelet count 140 G/L (range 150-400 G/L), prothrombin time was normal. Fibrinogen concentration was increased up to 0.69 g/L (normal range 0.2-0.45 g/L). There was a prolongation of the activated partial thromboplastin time (aPTT) up to 82.1 secs (normal range 26-40 secs), which did not correct following the in vitro addition of normal plasma. Acute disseminated intravascular coagulation (DIC) was excluded. The Factor VIII level was reduced to 0.6% (normal range 50%-150%). The Bethesda assay demonstrates an inhibitor 5.4 Bethesda Units (BU). He was screened for inflammatory and malignant disorder. Prostate specific antigen (PSA) rate was 400 ng/mL (normal range 0-4 ng/mL). Prostate cancer has been confirmed with multiple bone metastases. Treatment was aimed at stopping the acute bleeding, eliminating the inhibitor and curing the underlying disease. The patient was treated symptomatically with rFVIIa (NovoSeven) 270 µg/kg administered every 3-5 days due to diathesis intensity. Efficacy was judged by decreasing bleeding episodes. Anticancer therapy was ordered by urologist (flutamide and triptoreline). For eradication of the inhibitor 5.4 Bethesda Units (BU). He was screened for inflammato-
dcular coagulation (DIC) which was the only definite curative therapy in patients who have bleeding disorders and who died had factor V level below 10% (p<0.004) and had bleeding episodes of > 2 Conclusions-Factor V level on day 3 of diagnosis was in the range of 10%-25% and who died had factor V level below 10% (p<0.004) and had bleeding episodes of > 2 Conclusions-Factor V level on day 3 of diagnosis and episode of bleeding may help in predicting survival in patients of acute liver failure, thus help in triage for the need of orthotopic liver transplantation which is the only definite curative therapy in patients who have poor survival rates.

Thus we exploited the database collected by us in intensive care unit.

2. Aims-observational study to investigate major predictors of survival in viral hepatitis induced acute liver failure. Methods - observational study of 40 patients followed for 1 month to look for outcome-survival and death (7 days) or less and measurement of metabolic parameters like lactate and arterial blood gases and assessment of bleeding risk by carrying out coagulation factor level V which is synthesized in liver and not affected by vitamin K and factor VIII which is not synthesized in liver. Results-There were 12 patients who survived and had only 0-1 episode of mucosal bleeding. The analysis showed that patients who survived had factor V level on day 3 of diagnosis in the range of 10%-25% and who died had factor V level below 10% (p<0.004) and had bleeding episodes of > 2 Conclusions-Factor V level on day 3 of diagnosis and episode of bleeding may help in predicting survival in patients of acute liver failure, thus help in triage for the need of orthotopic liver transplantation which is the only definite curative therapy in patients who have poor survival rates.

1686

BLEEDING MAJOR SURVIVAL PREDICTOR IN PATIENTS OF VIRAL HEPATITIS INDUCED ACUTE LIVER FAILURE

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Background. In acute liver failure, variables favoring survival of patients is based on age, interval between onset of jaundice and encephalopathy, coagulation factor level, risk of bleeding but all these variables were concluded from the studies that were conducted in Western population where etiology most commonly is paracetamol poisoning and metabolic disorders like Wilson’s disease; whereas in Indian subcontinent set up major causative factor is viral hepatitis.

1685

FACTOR XIII DEFICIENCY IN ADULTHOOD - INHERITED OR ACQUIRED?

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The inherited deficiency of factor XIII is very rare (1 case per 2 million population) and is transmitted as an autosomal recessive disease. The clinical manifestations depend on the amount of factor circulating, ranging from severe bleeding since birth to just slow healing of tissues. Case report. Male, 40 years old, without previous known diseases, suffered muscle rupture one month before admission, with consequent formation of gluteal hematoma, which kept gradually growing. In this first month he was observed in different hospitals and developed progressive disseminated intravascular coagulation (DIC). He also underwent needle aspiration of the hematoma with subsequent clinical deterioration. At admission in our hospital he was pale but hemodynamically stable, with a right gluteal hematoma extending to the dorsal region and knee. He had anemia (10.5 g/dL), thrombocytopenia (75,000 x 10^6/L), decreased haptoglobin (<8 mg/dL) and fibrinogen (94 mg/dL). D-dimer increased (12.66 µg/mL) and slightly prolonged PT and aPTT. We then proceeded to the determination of coagulation factors, including the rarest, which showed a deficiency of factor XIII (11%; normal range 75-150%). The patient received transfusion of concentrate of this factor, resulting in reduction of the hematoma, resolution of DIC, and increase of haptoglobin. We were told by the patient that he had slow healing of the skin and small late bleeding since childhood. He was also submitted to an appendectomy four years before, without major bleeding or other problems. In the outpatient clinic he remained without hemorrhage, with Factor XIII between 50-70% without transfusion. Conclusions. The absence of both consanguinity in the family and prior major bleeding (including during surgery) suggested acquired deficiency. However, primary disease was excluded; also, the good response to the transfusion of the factor (suggesting inexistence of antibodies) and the presence of light but typical symptoms suggests inherited deficiency of factor XIII. In this case the deficiency was aggravated and only expressed in the context of DIC by trauma. The diagnosis of this entity allows the prevention of serious consequences, whether in the acute event or by performing prophylaxis. The transfusion of factor concentrate is effective and safe, without infectious complications.

1687

ASSESSING THE ORAL CONTRACEPTIVES EFFECTS ON THE COAGULATION AND FIBRINOLYSIS

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More than 100 million women in the world make use of hormonal contraceptives and, from these, 93 million use the oral combined hormonal contraceptive. In spite of its desired effect on contraception, its metabolization occurs at liver, stimulating the synthesis of the plasm-
ic proteins, among them, the ones that control the coagulation system and fibrinolysis. Initially, the estrogen present at the contraceptives was considered the sole responsible for the pro-thrombotic effects of hormonal contraception. However, with the continuous incidence of thrombosis induced by contraceptives containing low-concentrations of estrogen and different progestogens, it was possible observing that the hypercoagulant effect of the contraceptive was not only dose-dependent of estrogen. On this study, the alterations suggest that the fourth generation contraceptives have a smaller antiestrogenic activity in relation to the second generation one. In face of this, the purpose of this work was assessing the occurrence of hemostatic alterations on the Brazilian female population. The contraceptive used in this study was drospirenone (Yasmin®). This study contemplated 70 participants from the Health Center School of Ribeirão Preto Medical School - USP and from the Faculty of Pharmaceutical Sciences of Ribeirão Preto - USP. These volunteers have given their consent, on written form, for participating on this study, and they were distributed into four groups: a control group (20 patients) and three oral combined contraceptive ones containing ethinylestradiol 20µg and 30µg combined with 3000 µg drospirenone (16 and 20 patients, respectively) and ethinylestradiol 30µg combined with 150µg Levonorgestrel (14 patients). From these were assessed the following parameters: TP, Factor VII, TTPA, Factor XII, Fibrinogen, Factor I-2, Protein C, Protein S, Antithrombin, D-dimers and PAI-1. The contraceptive containing ethinylestradiol 20µg and drospirenone we've found alterations on the parameters TP, TTPA, Fibrinogen, Protein S and D-Dimers. For the formulation with ethinylestradiol 30µg and drospirenone we've found alterations on the parameters TP, Protein S and PAI-1. And, for the formulation containing ethinylestradiol 30µg and levonorgestrel we've found alterations on TP and on the Protein C concentration. In this way, the alterations suggest that the fourth generation contraceptive (ethinylestradiol 20µg and drospirenone) is among the assessed formulations, the medicine with the larger number of alterations associated to the hypercoagulability, whereas, the second generation contraceptive (ethinylestradiol 30µg and levonorgestrel) continues being the safest option to avoid the development of thrombosis, since it has presented the least tendency to hypercoagulability, confirming the fact that the pro-thrombotic alterations generated by the contraceptives don't depend strictly from the estrogen dose, but are strongly related to the type of progestogen.

### 1688 TREATMENT WITH FIBRINOGEN CONCENTRATE IN A PATIENT WITH SEVERE POSTPARTUM HAEMORRHAGE

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**Background.** Significant blood loss is a common complication of childbirth. Fibrinogen deficiency often results, and is exacerbated by large-volume transfusions of blood products or colloids/crystalloids (dilutional coagulopathy). Substitution with fibrinogen concentrate was considered the sole responsible for the hypercoagulability, and there are few reports of its use in obstetric hemorrhage. Here, we describe the use of fibrinogen concentrate, along with other haemostatic agents, in the successful management of a severe case of postpartum hemorrhage. **Aims.** To describe the use of fibrinogen concentrate in severe postpartum hemorrhage. **Methods:** In July 2010, a 32 years old woman, 39 weeks into her first pregnancy was admitted to the obstetrics department due to decreased foetal movements (day 1) and was decided to induce labour on day 2. During labour was determined to perform a caesarean section because of suspected acute foetal suffering and worsening of foetal bradycardia. In the post surgical unit she presented severe haemorrhage. Ongoing postpartum hemorrhage led to hypovolaemic shock and decrease of haemoglobin (8 g/dL, compared with 12 g/dL pre-admission) and also to hypotension and tachycardia. The patient presented uterine atony without response to conservative treatment, carried out with perfusion of oxytocin and sulphostone, as well as uterine compression. A second surgical plane was performed with subtotal hysterectomy and bladder repair, during which several transfusions were administered: nine units of erythrocyte concentrate, 8 mg of recombinant factor VII, 7 g of fibrinogen concentrate, six units of fresh frozen plasma, one platelet concentrate obtained by apheresis and 1000 IU of antithrombin III. During surgery the patient remained hypotensive and with conservative diagnosis, resulting in transfer to ICU. Sedation was administered and mechanical ventilation was implemented. During the hospitalization coagulation was not compromised in the puncture locals, but there was blood present in gastric drainage and microscopic haematoma was diagnosed. Patient was transfused with two units of erythrocyte concentrate due to anemia, although there were not evidences of haemostatic problem. Sedation and ventilation were withdrawn and extubation was performed without complications. Diuresis decreased, with some retention of nitrogen, resolved by fluid administration. Results: She was transferred to the obstetrics department (day 5) and was asymptomatic, received no further transfusions, and responded well to iron-saccharate treatment, haemoglobin level increased to 12.6 g/dL. On day 7, patient was discharged in good overall health. Haemodynamic stability was confirmed: blood pressure 100/60 mmHg, pulse rate 80 bpm. Haemostatic tests (prothrombin time, fibrinogen level, platelet count, haemoglobin level) were normal, as were renal function and respiration. In the follow-up, dextran was discontinued and came out negative, the analytical parameters were also normal. Both mother and baby remained healthy upon follow-up at 3, 6, 10 and 15 weeks. Results of laboratory haemostatic tests performed during the patient's hospital stay and follow-up are presented in the table. **Summary/Conclusions:** The successful outcome of this case can be attributed to effective haemostatic intervention, in particular, administration of fibrinogen concentrate was key to re-establishing haemostasis. Rapid, effective haemostatic therapy is vital for women with severe postpartum haemorrhage.

### 1689 EVALUATION OF THE ADMINISTRATION EFFICACY OF PROTHROMBIN COMPLEX: RETROSPECTIVE OF 4 YEARS OF USE IN A PORTUGUESE DISTRICT HOSPITAL

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**Background.** The human plasma-derived prothrombin complex containing coagulation factors II, VII, IX, X, Protein C and Protein S is indicated when rapid correction of haemostasis is necessary. It is used in patients with acute haemorrhage, mainly hypocoagulated patients, and in the correction of coagulation parameters in patients with hepatopathy, substituting fresh frozen plasma, as prothrombin complex improves haemostasis in a more rapidly way, without causing circulatory overload. **Aims:** The purpose of this work is to evaluate prothrombin complex in several pathologies during four years in a Portuguese district hospital. **Methods:** We performed a retrospective study of the use of the therapeutic in 172 patients, from 2007 to October 2010, in relation to gender, age, diagnostic, number of vials used, number of administrations, efficacy of the therapeutic and thrombosis occurrence three months after administration. **Results:** Average age was 67.4 years (15-91 years). Patients were treated in order to correct alterations of coagulation parameters. The first group was constituted by 135 (78.5%) hypocoagulated patients or patients with hepatopathy. From these, 39 (22.7%) were waiting for pre programmed surgery or pre invasive procedures, 59 (22.7%) had digestive bleeding, 23 (13.4%) had brain haemorrhage, 19 (11.0%) had the need of emergent surgery and 15 (8.7%) had thoracic and abdominal bleeding. The second group comprised 37 (21.5%) patients with coagulopathy: 16 (9.3%) were patients submitted to intestinal neoplasia surgery, 10 (5.8%) had bleeding caused by multiple trauma, 7 (4.1%) had bleeding caused by severe infection, 3 (1.7%) had post surgery uncontrollable hemarthria and 1 (0.6%) had post caesarean bleeding. The average number of vials used per patient was 2.5, each vial corresponding to 500 IU, and the average number of administrations was 1.2 times. The treatment was efficient in 152 (88.5%) patients. The patients that did not respond to the therapeutic died as a result of uncontrollable bleeding, multiorgan failure or fatal brain haemorrhage. Thrombotic events were not found in any patient treated with prothrombin complex after three months of treatment. **Summary/Conclusions:** Our experience revealed that prothrombin complex has an important role in the correction of coagulation parameters and control of haemorrhagic conditions in a rapid and efficient way, and without occurrence of thrombotic events.

### 1690 EPIDEMIOLOGY OF BLEEDING DISORDERS IN ZAGAZIG UNIVERSITY CHILDREN HOSPITAL, EGYPT: RETROSPECTIVE ANALYSIS

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**Background:** Bleeding defects are of great interest in pediatrics since the prevalence of congenital forms and the early appearance of acquired ones. They have been identified with a heterogeneous group of clinical disease that differs from one another in etiology, pathogenesis, epidemiology.
ology and incidence in population. Aims: to describe the epidemiological, laboratory, and clinical data of the various bleeding disorders observed in the Hematology Department of the Zagazig University Children Hospital, as based on retrospective analysis of clinical records between years 2006 and 2010. Patients and methods: The study included 279 children with bleeding disorders registered from 2006 to 2010 in Zagazig University Children Hospital. All cases with history of mucocutaneous bleeding or any other bleeding manifestations were subjected to full history and thorough clinical examination. Hematological profile (platelet count & function, bleeding time, PT, PTT and TT) and specific tests in congenital group (as coagulation factor assay and von willebrand factor assay) were done. Results: In this study bleeding disorders were significantly most common in males. Aplastic anemia especially coagulation disorders. Platelets disorders were found in 71.3% of our patients while coagulation disorders in 28.7%. In this study hemophilia A was the commonest coagulation disorders (58.7%) followed by hemophilia B (20%). Mild presentation represents the majority in 70% followed by moderate severity in 23.75%. We reported 2 cases of mild hemophilia A in females (6.9%) in our study. The initial clinical presentation of coagulation disorders was not significantly affected by sex or severity. The commonest complication of coagulation disorders found was hematrhosis. ITP was the commonest platelet disorders in our locality followed by aplastic anemia (45.9%, 22.6% respectively), 74.2% of patients with ITP were acute and 25.8% were chronic. The 5 years survival rate among patients with aplastic anemia was 69.3%, while the mortality rate was 31.7%. Conclusion: the most common bleeding disorders in our locality is ITP followed by aplastic anemia and hemophilia A. Attention must be given for early detection and accurate diagnosis of these disorders for appropriate management.

1691 THINK GLOBALLY, ACT LOCALLY - THE MEASURES OF LOCAL BLEEDING CONTROL IN PATIENTS WITH HEMOPHILIA AND INHIBITORS
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Background: By-passing agents made possible many invasive diagnostic or therapeutic procedures, in patients with hemophilia and high titer of inhibitors. But, these patients still have an increased risk of perioperative bleeding. In our institution, since January 1st, 2008. until January 1st 2011, there were 7 invasive procedures in patients with hemophilia and high titer of inhibitors. Two of them were major surgeries (limb amputation due to compartment syndrome and phlegmona, nephrectomy due to renal tumor causing constant macrohematuria) and five minor interventions (3 surgical, multiple tooth extractions, 1 diagnostic upper endoscopy and 1 upper endoscopy with hemoclipping and applicator of fibrin glue for rare bleeding gastric Dieulafoy lesion). We used recombinant factor VIIa (Novoseven en) or activated prothrombin complex (FEIBA). Former was used in doses of 90-270 mikrogr/BM every 2 hours, with gradual prolongation of intervals between doses and later was applied in doses of 50 IU per kg/BM every 6, 8 or 12 hours. Results: Both by-passing agents showed excellent efficacy, but local measures of bleeding control (fibrin glue, hemoclipping, compression packing and suturae) were very important. Only after nephrectomy, not a single measure of local hemostasis was performed, due to large bleeding surface. As a result we had single significant bleeding. Bleeding was stopped when the renal lodge was filled with blood, and pressures were equalized. Later, that blood collection was transformed into the pseudotumor, still shrinking one year after intervention. All our patients had improved performance status after the invasive treatment. Conclusions: Both by-passing agents showed great efficacy, but measures of local bleeding control seems to be equally important.

1692 INTRAMURAL HEMATOMA OF THE SMALL INTESTINE UNDER THE PROPHYLAXIS
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Intramural hematoma of the small intestine is a rare clinical condition. Spontaneous hematoma of the small intestine is seen in the warfarin overdose, and rarely in Hemophilia, ITP, vasculitis and malignancy. In this report, we will present a patient with Hemophilia developing small intestinal hematoma. A 25-year-old male patient who had been diagnosed since one year old as severe hemophilia was admitted to the emergency room with abdominal pain. He had a slight stomachache after meals for the last 2-3 days. A day before his admission, his abdominal pain got severe. After dinner on that day, he vomited. He was not suffering from fever, diarrhea or constipation. According to the laboratory results, The laboratory examination findings were as follows: white cell count: 10,500 (3,700-9,700), Hemoglobin: 15.9 (13.3-17.9) gr/dL, platelets: L, the level of Factor VIII: 1%, stool occult blood: positive; 250,000 / abdominal X-ray and abdominal ultrasound were normal. Magnetic resonance imaging (MRI) of terminal ileum was also normal; there was a 1 cm diffuse symmetrical thickening of the wall in a segment of about 15 cm long (Figure 1). Intestinal hematoma was diagnosed. Factor VIII concentrate was started. Dose was calculated for to raise factor level to %.40. After bolus dose of Factor, the level was checked as 55%. The patient’s symptoms decreased within 2-3 days. A week later, MRI was taken again and in the ileum showed that the thickness of the wall regressed (Figure 2). Control stool occult blood was negative. The incidence of the intestinal hematoma in patients with hemophilia is not known. By the use of tomography, imaging the number of diagnosed cases has increased. Treatment is usually conservative. Surgery is not required. Bleeding can be stopped with a strong factor replacement. Hemophilia patients with developed intestinal hematoma have been reported in the literature. We have presented a case that developed intestinal hematoma under the prophylaxis. Unfortunately, the reason for health insurance system in Turkey, our prophylaxis doses are limited with 4500 Units in a week. This dose is not reaching to doses recommending, especially in adult patient. He was taking 1000 Units three times in a week. Nevertheless, the bleeding episodes in joints were controlled with this dose. He started to study in court as archive employees since last year. There was hard working such as carrying heavy files in his recent history, especially last week. This experience reminded us, prophylaxis doses should be reevaluated in hemophilia patients regularly and abdominal pain can be herald of the life threatening bleeding.

1693 INTEGRATED POSTURAL ANALYSIS IN CHILDREN WITH HEMOPHILIA: A COMPARATIVE STUDY
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Background: integrated postural analysis represents a new approach to assess the presence of musculoskeletal alterations in children with
A 68 year old woman presented with prolonged activated partial thromboplastin time (APTT) who received orthopedic surgery. Level of FXII level was 4% while levels of factors VIII, IX, XI were decreased 41%, 31%, and 31% respectively. Lupus anticoagulants (LA) was excluded with DRVVT test (LA1 44.36, LA2 41.3 R=1.07). In mixing study with control plasma FXII insufficiently increased from 4% to 28% (control FXII 132%). Inhibitor to FXII detected with Bethesda like method shown level of 1.56 BU. This level of inhibitor correlates with ineffective increase of FXII with control plasma in mixing study. In next step clotting factors assayed at multiple dilutions. Factor XII appeared equally deficient no matter how much the patient plasma is diluted but other factors VIII, IX and XI increased over normal levels. This result strongly confirmed presence of FXII inhibitor. Also, we measured level of FXII in platelet reach plasma (PRP) before and after platelets lysis by freeze and heating. Levels were similar 4% and 6% respectively. All analysis indicated that patient have acquired deficiency of FXII due to inhibitors against FXII. Conclusion: Patient received anticoagulant prophylactic therapy during surgery and postoperatively during 35 days with low molecular weight heparin (LMWH) in dosage range for anti-FXa between 0.2 to 0.5. Presence of inhibitor to FXII was not associated with bleeding complication during the surgery and with thromboembolic complications after surgery that was prevented with LMWH postoperatively.

**1697**

**TRANSIENT ACQUIRED FACTOR VII DEFICIENCY AFTER LOBECTOMY PERFORMED FOR LUNG METASTATIC ADENOCARCINOMA: A CASE REPORT**

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A transient isolated deficiency of factor VII (FVII) is uncommon but has been described in association with infection, malignancy or inhibitor. Proposed mechanisms include proteases of neutrophils and macrophages and consumption associated with endothelial injury in sepsis; release of tissue factor (TF) from tumor; fixation of FVII to the tumor; increased concentration of tissue factor plasmatic inhibitor (TPII) produced by tumor and complex binding FIT–TPII, specific inhibitor (autoimmunity). We report a case the taking in charge of a patient with acquired FVII deficiency in the context of lobectomy for lung metastatic adenocarcinoma. The possible mechanism of FVII deficiency are discussed.
This case reports a patient with acquired FVII deficiency in the context of lung metastatic adenocarcinoma. Preoperative coagulation studies were normal and there was no personal or family history of a bleeding diathesis. Post-operative pulmonary infection with systemic sepsis occurred (procalcitonin: 3.72 ng/ml - N < 0.05 ng/ml, detection of Enterobacter aerogenes in bronchial aspiration). This sepsis was controlled by antibiotics including amoxicillin/clavulanic acid and levofloxacin. Two days after surgery, haemostasis tests showed prolonged prothrombin time corrected by mixing test. Further investigations brought out isolated FVII deficiency. Bethesda test to assess inhibitor activity was negative. Plasma levels of liver enzymes were normal and vitamin K administration did not normalize the prothrombin time. To prevent bleeding during chest drains removal, the patient received recombinant activated FVII (epoetacog-alpha, 1mg, 13.8 µg/Kg). Calibrated Automated Thrombinography (Diagnostica Stago) assay showed a longer lag time than plasma control and injection of recombinant activated FVII shortened the lag time without changing endogenous thrombin potential. Systematic biological control revealed a spontaneous normalization of coagulation tests in five days. This case report displays a conjunction of several possible aetiologies of transient acquired FVII deficiency, namely sepsis, malignancy and FVII inhibitor. It should be noted the presence of lung metastatic adenocarcinoma did not lead to FVII deficiency before surgery. With the Bethesda test, we could exclude the appearance of a FVII inhibitor. Although it was rapidly controlled by antibiotics, sepsis may lead to a derangement of granulocyte-associated proteases that cleave FVII and to an overconsumption of FVII consecutive to damaged vascular endothelium. Finally, the transient acquired FVII deficiency could be linked to an increased FVII clearance by circulating tissue factor or tissue factor pathway inhibitor released during tumor resection surgery.

Factor XI deficiency epidemiology in Iran

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Factor XI deficiency categorized as a rare bleeding disorder with several clinical manifestations in patients. Aims: This review gives an overview of the epidemiology, clinical manifestations, and bleeding severity in Iranian factor XI deficiency patients. The correlation between FXI levels and the bleeding tendency is much less clear than in the hemophilia, and consequently the bleeding risk can be difficult to predict. Methods: In this study we have used a questionnaire of granulocyte-associated proteases that cleave FVII and to an overconsumption of FVII consecutive to damaged vascular endothelium. Finally, the transient acquired FVII deficiency could be linked to an increased FVII clearance by circulating tissue factor or tissue factor pathway inhibitor released during tumor resection surgery.

Factor V Leiden is the main etiological factor in Egyptian patients with Budd-Chiari syndrome

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Factor V Leiden is the main etiological factor in Egyptian patients with Budd-Chiari syndrome. Methods: The study included 47 (20 children and 27 adults) patients with Budd-Chiari syndrome. Genotyping of factor V Leiden C677T, Prothrombin G20210A and Methyltetrahydrofolate reductase C677T were performed by real time PCR using fluorescence melting curve detection analysis by means of the Light Cycler System. Results: Factor V Leiden was present in 28 patients (59.6%) which was significa-
cantly higher compared to previous reports. It could be the sole factor causing Budd-Chiari syndrome in 19 patients and in 5 patients with inferior vena cava involvement. Myeloproliferative disease was present in only 10 (21.3%), antiphospholipid syndrome in 5 (10.6%) and Behcet disease in 3 patients (6.4%). Interestingly, 3 children with lipid storage disease had Budd-Chiari syndrome. Conclusions: Factor V Leiden could be considered as the main etiological factor for Budd-Chiari syndrome in Egyptian patients which could be attributed to the geographical distribution of this mutation in this area. Its sole presence could be considered as a strong thrombophilic factor and a leading cause of inferior vena cava thrombosis in these patients. Lipid storage disease should be included as a risk factor for this disease.

**1701**

**STUDY OF URINARY 11-DEHYDRO-THROMBOXANE B2 AS A MARKER OF THROMBOEMBOLIC EVENTS IN β-TALASSEMAIA PATIENTS**

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**Introduction:** There is increasing evidence that chronic hemolytic anemia such as β-thalassemia (BT) is characterized by a hypercoagulable state. In addition to increased thrombin and fibrin generation, increased tissue factor activity, and increased platelet activation, patients with hemolytic anemia manifest thrombotic complications, including venous thromboembolism and stroke. Aims: To evaluate the role of platelets activation by measuring the urinary level of 11-dehydro-thromboxane B2 as a marker of thromboembolic events in BT patients. Subjects: This study was carried out in at thalassemia outpatient clinic at Zagazig university hospitals. The study included a total number of 50 patients and 20 healthy controls, classified into: Group I: Included 20 healthy and haematomally normal subjects as a control group. Group II: Included 20 non-splenectomized β-thalassemia major (NSBTM) patients. Group III: Included 18 β-thalassemia intermedia (NSBTI) patients. Group IV: Included 15 splenectomized β-thalassemia major (SBTM) patients. Methods: All the patients and controls were subjected to the following: history taking and clinical examination, hemoglobin electrophoresis, prothrombin time (PT), Partial thromboplastin time (PTT), urinalysis, D-dime, fibrinogen, brain MRI and Doppler ultrasound on the abdomen. The estimation of 11 dehydro-thromboxane B2 level was done by ELISA from DRG international company provided by clinilab and the results were reported as pg urinary 11dhydroTxB2 per ml urinary creatinine in order to normalize results for the concentration of the urine sample tested. Results: In this study Urinary 11 dehydro TxB2 pg/mg creatinine revealed very high significant increase in the all groups compared to that of the control group. In addition, there were significant difference between the patients group II and group III and IV (P<0.05), while there were no significant increase between group III & IV (P>0.05). Discussion: There was a strong positive correlation in this study between 11 dehydro-TXB2 and D-dimer level in groups II, III and IV. On the other hand there were strong negative correlation between 11 dehydro-TXB2 and fibrinogen level in groups II, III and IV. In this results, no significant correlations were found between platelets counts and hypercoagulability markers (11 dehydro-TXB2, D-dimer and fibrinogen) in all studied groups (P>0.05). A strong inverse correlation was found between HB F and both 11 dehydro TXB2 and D dimer in group II, III and IV. Conclusion: The elevated urinary 11-dehydro-TXB2 level can be used a marker of hypercoagulable state even in clinical asymptomatic thalassemic patients.

**1702**

**RETROSPECTIVE ASSESSMENT OF RISK FACTORS FOR VTE IN ADOLESCENTS OF SOUTH MORAVIAN REGION, CZECH REPUBLIC WITHIN YEARS 2004-2010**

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**Background:** Venous thromboembolism (VTE) is multifactorial disease. There are many known risk factors (RF) for VTE, both acquired and inherited. VTE is relatively infrequent in children and adolescents compared to adults. Aims: To analyse RF of VTE in adolescents. Methods: We retrospectively analysed RF for VTE in consecutive adolescent patients treated in regional referral centre for their first VTE episode in years 2004-2010. The patients were 14 to 18 years old. Analysed parameters were as follows: age; sex; thrombus location; pulmonary embolism (PE) and respective RF- both inherited (antithrombin (AT) deficiency; protein C (PC) deficiency; protein S (PS) deficiency; FV Leiden mutation; FVL mutation; protrombin gene mutation (FII G20210A); high FVIII levels; protein C global test (PCG) positivity and family history positive for VTE) and acquired (obesity, smoking, lupus anticoagulant, surgery, knee arthroscopy, immobilisation, pregnancy, delivery, abortion, autoimmune disease and oestrogen-containing oral contraception (COC)). We excluded central venous line (CVL) related VTEs as well as other non-VTE thrombotic events from our cohort. Results: Seventy-two consecutive patients were analysed for their first VTE (46 female (63,9%) and 26 male (36,1%). Median of their age was 16 years, ranging from 14 to 18. Thrombus location in this cohort of patients was as follows: DVT of lower extremities and pelvis 56 (77,5%), DVT of upper extremities 8 (11,1%), one (1,4%) thrombotic episode of hepatic veins, one (1,4%) thrombosis of internal jugular vein and 2 (2,8%) thrombosis of cerebral veins. In 4 (5,6%) patients with PE we were not able to identify the location of primary thrombosis. The total number of patients with PE was 8 (11,1%). One patient died due to PE. Prevalence of respective RF was: positive family history 11 (15,5 %), FVL 20 (27,8%), FII G20210A 4 (5,6%), deficiency of AT 3 (4,2%), PC 3 (4,4%), PS 6 (8,8%); isolated positivity of PCG 11 (15,7%), FVIII high 20 (45,4%), vessel pathology 3 (4,2%), obesity 2 (2,8%), smoking 6 (8,8%), knee arthroscopy 7 (9,7%), LA positivity 9 (12,7), other acquired RF 36 (50%). COC 30 (47,6% from all, 65,2% of girls). In median, our patients had 2 RF (range 0-6 RF/patient). Only one patient (1,4%) revealed very high significant increase in the all groups compared to that of any known RF. Twelve patients (16,7%) had only inherited RF, 23 (31,9%) had only acquired RF and 36 (50%) of patients in our cohort had combination of both inherited and acquired risks. We estimated the overall incidence of DVT (non CVL related) in age group of 14 to 17 years old adolescents in our region to be minimally 16/100000/year (2004-10); in females minimally 21/100000/year and in males 10/100000/year. Conclusions: Incidence of VTE in our cohort is relatively high (compared to published data), higher then that in children and closer to that in adults. VTE form thus a real threat for Czech adolescents. Less than 20% VTE episodes in our cohort were idiopathic/unprovoked. Prevention of VTE is necessary in this age group, and should be based mainly on recommendations for adults. Further prospective studies are needed to confirm our findings on a nation-wide basis.

**1703**

**POSSIBLE INFLUENCE OF ACQUIRED AND INHERITED THROMBOPHILIC FACTORS ON PORTAL VEIN THROMBOSIS**

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**Aims:** The aim of this study was to explore the prevalence of local and genetic thrombophilic disorders as risk factors for portal vein thrombosis (PVT) in our pediatric series. Methods: We conducted a case-control study enrolling 35 children with PVT and 26 age-matched controls. All were screened for thrombophilia, including genetic disorders, protein C, protein S and homocysteine deficiencies. All coagulation parameters were studied in all, 65,2% of girls). In median, our patients had 2 RF (range 0-6 RF/patient). Only one patient (1,4%) revealed very high significant increase in the all groups compared to that of any known RF. Twelve patients (16,7%) had only inherited RF, 23 (31,9%) had only acquired RF and 36 (50%) of patients in our cohort had combination of both inherited and acquired risks. We estimated the overall incidence of DVT (non CVL related) in age group of 14 to 17 years old adolescents in our region to be minimally 16/100000/year (2004-10); in females minimally 21/100000/year and in males 10/100000/year. Conclusions: Incidence of VTE in our cohort is relatively high (compared to published data), higher then that in children and closer to that in adults. VTE form thus a real threat for Czech adolescents. Less than 20% VTE episodes in our cohort were idiopathic/unprovoked. Prevention of VTE is necessary in this age group, and should be based mainly on recommendations for adults. Further prospective studies are needed to confirm our findings on a nation-wide basis.

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**1704**

**SETTING A TARGET INTERNATIONAL NORMALIZED RATIO RANGE DOESN’T GUARANTEE GOOD ACHIEVEMENT OF THAT RANGE-A STUDY IN OMANI PATIENTS TAKING WARFARIN**

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**Background.** Warfarin is a worldwide oral anticoagulant commonly used to prevent thrombosis, and international normalized ratio (INR) is used to monitor the dose of warfarin. INR is usually set either in the range of 2.5-3.5 dependent on the underlying disorders. Aims: To know the actual achievement of target INR in two groups of patients whose INR ranges were set either in 2.5-3.5 or 2.5-3.5. Methods: From September 2010 to January 2011, we consecutively enrolled 131 patients who were taking warfarin because of thrombosis, atrial fibrillation or mechanical heart valves (MHVs) and were regularly followed up in our specialist clinics. We reviewed their history, recorded the diagnosis, INR data and warfarin dosages. Two groups of patients were divided, Group A included patients with MHVs, the target INR range was set into 2.5-3.5; Group B included patients other than MHVs, and the target INR range was set into 2.3. We compared the percentage of time which was within the target INR ranges in these two groups. Results: Totally 113 patients, with 1976 results of INR were enrolled. The total follow-up period was 188.4 patient-years. Group A included 29 patients, their mean age was 45.4 ± 16.0 years, mean INR was 2.96 ± 0.61, and mean warfarin dose was 4.69 ± 2.08 mg. Group B included 88 patients with mean age 52.7 ± 16.7 years which was significantly older than that in Group A (p = 0.007). Their mean INR ± SD was 2.67 ± 0.16, which was significantly lower than that of Group A (p = 0.001); their mean warfarin dose ± SD was 4.80 ± 2.44 mg, which was not different from that of Group A (p = 0.215). 154 (32.8%) INRs were within the target range of 2.5-3.5 in Group A, whereas 333 (55.4%) INRs were within the target range of 2-3 in Group B. Interestingly, although the target INR in Group A was set in the range of 2.5 to 3.5, 187 (39.8%) INRs were found in the range of 2-3, which was more than those in the range of 2.5 to 3.5 (39.8% vs 32.8%) in the same group. Moreover, the percentage of INR range from 2-3 in Group A, which was originally set with INR range of 2.5-3.5, was more than the percentage found in Group B (39.8% vs 35.4%) whose INR range was initially set in 2-3. Thus, although the mean of INR in Group A was significantly higher than that in Group B, the percentage of INR ranging from 2-3 was higher in Group A than in Group B. i.e. the original setting of INR target with frequent monitoring could not result in a higher rate of that target. Conclusions. Simply setting the target range of INR by laboratory test in a specialist clinic is not enough to achieve good INR targeting. Many other causes would influence the achievement of the target INR. As this might be a common phenomenon, attention to this problem with more studies to find out a way to solve this problem is urgently necessary.

**1706**

**WARFARIN LOADING & DOSING AT A DISTRICT GENERAL HOSPITAL**

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**Background.** Warfarin is a Vitamin K antagonist. It is a synthetic derivative of dicoumarol, a fermentation product of coumarin. In 1940 dicoumarol was isolated at University of Wisconsin. It was the first drug in its class, patented in 1941. Warfarin is a combination of Wisconsin Alumni Research Foundation (W.A.R.F) and -arin indicating the link with coumarin. Warfarin was first registered for use as a rodenticide in the US in 1948. In 1954 Warfarin was first approved for medical use for humans as an anticoagulant. International Normalized Ratio (INR), is a system established by the W.H.O. and the International Committee on Thrombosis and Haemostasis for reporting the results of blood coagulation tests. Objectives. The goal of this audit was to look at the Warfarin loading and dosing regimes of patients at our institution between January 2009 and January 2010. We wished to compare our findings to the National standards, and provide evidence for the development of better standards of INR monitoring especially during the loading phase. Our standards included - 1) All patients loaded on Warfarin should reach their target INR with in 5 days of starting, as in keeping with Fennerty et al. findings. 2) INR checked daily during loading process. 3) Warfarin loaded according to hospital protocol. Methods. A Retrospective audit measuring against the BCSH and local guidelines was performed. The inclusion criteria for this audit required patients to be loaded on Warfarin during their inpatient stay in the year 2009. Based on this criterion, a number of elements were extrapolated, including: - Age/gender ratio, reason for admission, reason for Warfarin loading, Anticoagulation prior to admission, INR on admission, INR at first loading dose of Warfarin, INR checked daily, number of days to target INR, loading dose, INR checked daily, whether patient was on Heparin during Warfarin loading, are referral letters to the anticoagulation clinic present, and if the referral letters to the anticoagulation clinic complete.

**1705**

**A COMPARATIVE EVALUATION OF SERUM FERRITIN LEVELS IN PATIENTS WITH DIABETES MELLITUS AND CHRONIC RENAL DISEASE WITH AND WITHOUT DEFECTS OF DIABETIC RETINOPATHY**

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**Background.** It has been hypothesized that the diabetes mellitus type 2 related microangiopathy may be associated with elevated absorption and storage of dietary iron represented by increased serum ferritin levels. Aims: To comparatively evaluate the levels of serum ferritin in diabetic patients with and without diabetic retinopathy lesions and the healthy controls (p = 0.010 and p = 0.006 respectively), while no significant difference in the values between the two groups of diabetic patients was found (p = 0.704). Moreover, there was no significant correlation between ferritin values and the values of HbA1c in both groups of diabetic patients (p = 0.365 and p = 0.941 respectively). Summary/conclusions: The results show that the levels of iron in diabetic patients may play a role in the occurrence of microangiopathy or its evolution.
Antithrombotic drugs. Results. MP concentration (nM) in patients before and with or without stent implantation.

Using ZYMOPHEN MP-Activity test before, 24 h and 1 month after PCI. Evaluation has been performed in 32 pts. MP concentration was measured in 73 pts (44 men, 29 women) with CAD. During PCI stent implantation and in men 24h after stent implantation.

INTERVENTION ON THE PLASMA MICROPARTICLES CONCENTRATION

The study group consisted of 25 patients (18 women and 7 men) of the mean age of 46 years (20 - 75 years). Concomitantly 9 of them had another autoimmune disorders and 4 had neoplasms. Among risk factors for arterial thrombosis 5 patients had hypercholesterolemia, 5 - hypertension, 4 were smokers and 4 were obese (BMI >30 kg/m2). None of the patients had hereditary thrombophilia (antithrombin, Protein C or protein S deficiency, factor V Leiden, prothrombin G20210A mutation, increased activity of factor VIII). Family history of venous thromboembolic disease has been noticed in 4 patients and of arterial thrombosis in 6 patients. The observation lasted for 5 to 127 months (mean 35 months). The number and percent of patients (n = 25) with abnormal results of different laboratory diagnostic assays for APA: Assessment of Lupus coagulant test including a dilute APTT and a dRVVT followed by anti-beta2-glycoprotein I(GPI) antibodies [by ELISA], along with a panel of microparticles concentration in the plasma of patients (pts) with coronary artery disease (CAD) taking aspirin and clopidogrel, before as well as after elective percutaneous coronary intervention (PCI) with or without stent implantation. Methods. The study group consisted of 73 pts (44 men, 29 women) with CAD. During PCI stent implantation has been performed in 32 pts. MP concentration was measured using ZYMOPHEN MP-Activity test before, 24 h and 1 month after PCI. In the control group there were 24 healthy blood donors not taking antiplatelet drugs. Results. MP concentration (nM) in patients before and after PCI is listed in attached table. Result of the control group: 4.6 nM +/- 3.3; VC 72%.

CONCLUSIONS

Reduced sample. 41 % reached their target INR with in 5 days. 18% of patients had an INR check on a daily basis. 11% of patients were loaded according to the hospital protocol that is based on the Fennerty et al study presented in the BMJ in 1984. The study was to register venous and/or thrombotic events in a group of patients with asymptomatic APA, diagnosed according to the international guidelines (Myakis et al. 2006). The study group consisted of 25 patients (18 women and 7 men) of the mean age of 46 years (20 - 75 years). Concomitantly 9 of them had another autoimmune disorders and 4 had neoplasms. Among risk factors for arterial thrombosis 5 patients had hypercholesterolemia, 5 - hypertension, 4 were smokers and 4 were obese (BMI >30 kg/m2). None of the patients had hereditary thrombophilia (antithrombin, Protein C or protein S deficiency, factor V Leiden, prothrombin G20210A mutation, increased activity of factor VIII). Family history of venous thromboembolic disease has been noticed in 4 patients and of arterial thrombosis in 6 patients. The observation lasted for 5 to 127 months (mean 35 months). The number and percent of patients (n = 25) with abnormal results of different laboratory diagnostic assays for APA: Assessment of Lupus coagulant test including a dilute APTT and a dRVVT followed by anti-beta2-glycoprotein I(GPI) antibodies [by ELISA], along with a panel of microparticles concentration in the plasma of patients (pts) with coronary artery disease (CAD) taking aspirin and clopidogrel, before as well as after elective percutaneous coronary intervention (PCI) with or without stent implantation. Methods. The study group consisted of 73 pts (44 men, 29 women) with CAD. During PCI stent implantation has been performed in 32 pts. MP concentration was measured using ZYMOPHEN MP-Activity test before, 24 h and 1 month after PCI. In the control group there were 24 healthy blood donors not taking antiplatelet drugs. Results. MP concentration (nM) in patients before and after PCI is listed in attached table. Result of the control group: 4.6 nM +/- 3.3; VC 72%.

Conclusions. 1. Mean microparticles concentration in the plasma of patients with coronary disease taking aspirin and clopidogrel is higher than controls, but SD is high, therefore the difference is not statistically significant. 2. There is an increase of microparticles concentration 24h after percutaneous coronary intervention in women without stent implantation and in men 24h after stent placement.

PREVALENCe, SPECTRUM AND CORRELATION OF LUPUS ANTICOAGULANT POSITIVITY WITH CLINICAL OUTCOME: EXPERIENCE FROM A SINGLE TERTIARY CENTRE IN OMAN

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Background. Lupus anticoagulants (LA) are immunoglobulins which prolong phospholipid-dependent coagulation tests. These inhibitors recognize anionic phospholipid epitopes and prevent or delay the generation of phospholipid dependent activation complexes in vitro. However, in vivo, they are associated with endothelial injury that alters the fibrinolytic balance leading to vascular micro-infarcts which clinically manifest as thromboembolism, thrombocytopenia and recurrent pregnancy loss. Aims. To study the prevalence and spectrum of antiphospholipid antibody (LA) positivity diagnosed using the 2009 ISTH criteria and correlate it with one of the three clinical outcomes namely thrombosis, thrombocytopenia and recurrent pregnancy loss. Methods. One hundred forty-seven consecutive patients (Males=47; Females=100) with mean age of 30.5 years [SD±17.1; Range 1-82 years] and an abnormal APTT that did not correct on mixing studies were retrospectively analyzed. Blood was collected for a complete blood count and serum and plasma samples were tested for IgG and IgM anticardiolipin (ACA) and anti-beta2-glycoprotein (β2GPI) antibodies [by ELISA], along with a panel of Lupus coagulant test including a dilute APTT and a dRVVT followed by mixing and confirmation studies with normal plasma and hexagonal phospholipid reagent respectively. 4. Results: Out of 147 cases, 146 cases with a positive LA, 48 (32.9%) had SLE (primary-25, secondary-25); 26 (17.8%) had thrombosis (DVT-12, Stroke-11, PE-1,MI-1, Portal vein thrombosis-1); 25 (17.1%) had thrombocytopenia; 20 (13.7%) had APLS (primary-8, secondary-12), and 13 (8.9%) had pregnancy loss (Recurrent miscarriage-11, IUPD-2). Incidentally, one patient had an acquired factor V inhibitor with severe factor V deficiency (FV<1%). The overall prevalence of ACA and anti-beta2 GPI antibodies was 17.1% and 17.8%, whereas, in patients with SLE it was 2-fold higher at 31.3% and 29.2%, but in patients with APLS it was 4-fold higher at 70% each respectively (p<0.05; Chi Square test). Furthermore, there was a good correlation between the ACA and anti-beta2 GPI antibodies (Pearson’s correlation coefficient r=0.53; p<0.05). 5. Conclusions. Antiphospholipid antibodies are heterogeneous, with LA positivity 5-fold more prevalent as compared to ACA or anti-beta 2 GPI antibodies. However, there was a good concordance between ACA and anti-beta 2 GPI antibodies positivity in the LA positive cohort. Furthermore both these antiphospholipid antibodies were equally prevalent in primary vs secondary SLE and APLS patients.
**1710**

DETECTION OF MUTATIONS IN THE ATIII GENE IN PATIENTS WITH VTE AND ATIII DEFICIENCY

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Antithrombin III (ATIII) is a 58kd protein synthesized in the liver that belongs to the family of serpins. It inhibits thrombin by binding to it and forming a stoichiometric complex. The velocity of the complex is greatly accelerated in the presence of heparin (>1000 fold). ATIII also inhibits the action of factors IXa, Xa, X, and Xla and the TF/VIIa complex. Its deficiency predisposes to recurrent VTE episodes. Normal levels range from 70-120%. It is inherited in an autosomal dominant manner, and has been identified only in the heterozygous state, suggesting that its total absence is incompatible with life. Homozygosity has been described in mutations that involve the heparin binding site. It is found with a frequency of 1% to 2% of VTE patients. The gene, located on chromosome 1q25.1, is 13.5 kb long, consists of 7 exons and encodes for a 482k amino acid protein. Various mutations have been described, including nonsense, non-nonsense, splice-site mutations, frameshifts, insertions and deletions. The functional (ATIII: C, mean 46%, range 19-62%) and antigenic (ATIII:Ag, mean 58%, range 35-113%) levels of ATIII were measured in VTE patients (n=30) with ATII deficiency. We present preliminary results of the detection of ATIII mutations. DNA was isolated from whole blood and exons and intron-exon boundaries were amplified. Multiplex PCR was also performed, whereby the 7 exons of the ATIII gene are simultaneously amplified, along with an internal quality control. All products were analyzed using dhPLC. PCR products were denatured and then gradually frozen to form heterodimers (detection of point mutations, small deletions and insertions) that are separated by the WAVE system. In cases where the chromatograms showed presence of heterodimers, samples were further analyzed by sequencing. The multiplex PCR products (detection of large deletions in heterozygous form that cannot be otherwise detected due to the presence of one normal allele) were also separated by the WAVE system and compared to the chromatograms of healthy subjects. The presence of lower peaks in the patient samples suggested the detection of exons. Up to date, 10 patients have been examined. 3 were found to have the Budapest III mutation in exon 2 of the gene, leading to substitution of a cysteine by thymine (C to TCC), resulting in the substitution of a leucine by a phenylalanine (L131F). This is thought to change the isolectic point of the protein that perturbs the geometry of the positively charged surface at the heparin binding site resulting in a low affinity for heparin. A frameshift mutation was also identified in exon 2 of one of the patients, as well as point mutations that result in a non-synonymous change of the amino acid and in premature stop codons (exons 1, 4, 5 and 6 in particular W189R, Q334X, D342G, R359X). To our knowledge, this is the first documentation of mutations in the ATIII gene in Greece.

**1711**

PLASMA COBALAMIN, FOLATE, PYRIDOXINE AND HOMOCYSTEINE LEVELS AND THROMBOSIS IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS AND HEMOLYTIC ANEMIAS

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Background. Hyperhomocysteinemia is a condition that may cause both arterial and venous thrombosis. In the etiology of hyperhomocysteinemia there are a lot of factors including folate, cobalamin and pyridoxine deficiencies. It has been shown that in chronic myeloproliferative neoplasms (MPNs) and chronic hemolytic anemias (CHA), the risk for thrombosis is higher than the normal population and plasma folate and cobalamin levels are significantly lower than the normal individuals. However there are small number of studies investigating the homocysteine (Hcy) levels in MPN and CHA patients. Aims: In this study, our aim was to investigate the thrombosis incidence, the effects of plasma total homocysteine (Hcy) levels on thrombosis formation and the correlation between folate, cobalamin, pyridoxine and homocysteine levels in MPNs and CHAs and to compare the results with the healthy control group. Methods: 41 patients [MPN (n = 31) and CHA (n = 10)] and 40 age and sex matched healthy individuals representing the control group were included in the study. For all the objects plasma tHcy, folate, cobalamin, pyridoxine levels were measured, and bilateral lower extremity venous system B-Mode and colour Doppler compression ultrasound imaging was performed to investigate the deep venous thrombosis (DVT). All the patients and the objects in the control group were questioned for the past arterial or venous thrombosis and for the comorbidities that may cause thrombosis. Also for each object smoking habits was taken and recorded as package/year. The follow up durations were noted for each object as months. In addition we measured the D-dimer levels to exclude acute thrombosis and also investigated the thrombophilic disorders other than hyperhomocysteinemia in the objects with thrombosis. Results. 11 thrombotic events in 7 objects were detect in the control group. Statistically significant and positive correlations were found between the follow up duration, comorbidity and thrombosis (r=0.455 and 0.248 respectively). There were no statistically significant correlations between the plasma tHcy and vitamin levels and thrombosis. In the MPN group, there were no statistically significant correlations between hematocrit, platelet counts and thrombosis. A statistically significant and negative correlation between plasma tHcy levels and folate (r=−0.312) also a statistically significant and positive correlation between tHcy and the amount of smoking (r=0.310) were detected. Conclusions. In conclusion, comorbidity may be an important factor in the etiology of thrombosis in patients with chronic myeloproliferative neoplasms and CHA patients. Also we think that in these patients in order to keep the plasma tHcy levels low, folate replacement therapy and reducing smoking are the treatment modalities that may prevent thrombosis formation.

**1712**

THE EFFECT OF INFLAMMATORY CYTOKINES ON VON WILLEBRAND FACTOR SYNTHESIS

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Background. Upon vascular injury, during the early stage of systemic inflammation, endothelial cell stimulation lead to the secretion of a family of monocyte-derived peptides, which include the cytokines interleukin-6 (IL-6), IL-8 and tumour necrosis factor-alpha (TNF-α). These cytokines stimulate the endothelial release of Ultra large Von Willebrand factor (ULWVF) multimers. Increasing VWF multimer levels may have inhibitory effects on the synthesis of the ULWVF cleaving enzyme, ADAMTS-13. This may ultimately lead to the deficiency of ADAMTS-13 during inflammation and the over expression of ULWVF multimers resulting in the initiation of thrombotic thrombocytopenic purpura (TTP). Furthermore, the role of the initiation of coagulation in TTP is not known. Aim: In this study, our aim was to examine the effects of inflammatory cytokines and coagulation factors such as tissue factor and thrombin on the release of ULWVF by cultured endothelial cells and the role of these ULWVF by ADAMTS-13. This will allow us to evaluate potential links between inflammation and thrombosis and help us understand the mechanisms that lead to TTP in patients. Methods: Human umbilical vein endothelial cells (HUVEC) were treated with interleukin 6 (IL-6), IL-8 and tumour necrosis factor α (TNF-α) for 24 hours under static conditions. The cells were then assembled to form the bottom of the flow chamber and exposed to a shear stress of 2.5 dyn/cm2 to expose VWF cleaving sites. The Von Willebrand factor secretion was then measured through an ELISA technique. Results. IL-8 and TNF-α stimulated the release of VWF after 24 hours of treatment while IL-6 did not induce the release of VWF due to the absence of the IL-6 receptor on HUVEC cells. Conclusions. These results suggest that inflammatory cytokines may stimulate VWF release. Further research will involve measuring ADAMTS-13 levels and activity after stimulation with the inflammatory cytokines as well as coagulation atorinitiators, thrombin and tissue factor. The combined effect of specific cytokines with coagulation initiators will be tested to look at the possibility of enhanced secretion of VWF or inhibition of ADAMTS-13. The findings might describe a potential linkage between inflammation and thrombosis.

**1713**

ENDOTHelial MICROPARTICLES: A POSSIBLE ROLE IN INFLAMMATION AND THROMBOSIS

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Background. Microparticles are vesicles formed from activation of cells. These particles have an effect on a number of disorders including inflammation, diabetes mellitus, atherosclerosis, thrombocytopenia,
cardiovascular and other cellular disorders. Elevations of microparticles might be an indication of cellular dysfunction and may thus be useful as a diagnostic marker. Microparticles can also be used as therapeutic targets in treatment of certain diseases, since these particles transports proteins and signals across cell membranes. Inflammatory cytokines and thrombotic stimuli are responsible for activation of cells and mediate microparticle formation. Von Willebrand factor is expressed on endothelial microparticle membranes upon activation of endothelial cells. This may indicate an important role for microparticles in primary clot formation in circulation. Aim The aim of the study was to determine the effect of inflammatory cytokines (Tumour necrosis factor α, Interleukin 8 and Interleukin 6) on human umbilical vein endothelial cell composition and its effect on thrombosis and von Willebrand factor in vitro. Methods Human umbilical vein endothelial cells were cultured in sterile conditions. Specific concentrations (0μM and 100μM) of inflammatory cytokines (tumour necrosis factor α, Interleukin 8 and Interleukin 6) were used to stimulate endothelial cells (HUVEC) in static conditions for 24 hours. Treated cells and untreated controls attached on the flask were irrigated in round petri dishes for half an hour on a horizontal shaker to create a shear stress of 2.5dyn/cm2. Microparticles were isolated by a Ceveron microparticle filtration unit. Thrombin generation assays were performed and von Willebrand factor antigen levels were measured using only the isolated microparticles. Results Thrombin was generated and von Willebrand factor levels were increased with both inflammatory and thrombotic stimuli. Conclusions Human umbilical vein endothelial derived microparticles have been illustrated to increase the von Willebrand factor levels from untreated to treated cells. Endothelial microparticles also generated thrombin after inflammatory stimulation of cells. This suggests that microparticles might have an effect on thrombin generation in locations other than the static endothelial cell environment, where inflammation or thrombosis may occur. Continued studies on von Willebrand factor activity and the metalloprotease of von Willebrand factor, ADAMTS-13 will be conducted to determine the effect of endothelial microparticles on the breaking down of ultra large von Willebrand factor multimers. This will give more insight on the effect of endothelial microparticles in inflammation and thrombosis.

1714
ACQUIRED ACTIVATED PROTEIN C RESISTANCE IN PATIENTS WITH SARCOMA

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Background: Although the relationship between cancer and thrombosis has been known for long years and the pathogenesis has partially been understood, the relationship between sarcoma and thrombosis has been reported recently and detailed information about its pathogenesis is lacking. Aim The aim of this study was to determine the frequency of clinically detectable thrombosis and presence of acquired activated protein C resistance (aAPCR) in patients with newly diagnosed sarcoma. Methods: We detected the cases who developed aAPCR prospectively in 52 patients (in the period after the diagnosis and treatment) and 52 healthy controls. We also determined the Factor (F) V and FVIII levels. Results: While thrombosis was significantly increased following treatment (median 94.35%), it was found to be significantly lower compared to that of the control group (median 8.38%, p<0.05, p<0.001 and p<0.001, respectively). aAPCR was found as 14% of patients at the time of diagnosis (13% versus 4.2%, totally disappearing after treatment. Thirdly, we found out that the significantly higher rate of aAPCR at the time of diagnosis (13%) and at the end of treatments (T2), TAT levels returned to pre-treatment values. PAI-1 levels remained significantly elevated from T0 to T1, occurred in subjects given LMWH, but not in those receiving placebo (p<0.05). After discontinuation of the study drug (T2), TAT levels remained to pre-treatment values. PAI-1 levels remained significantly higher basal levels of vWF than those without (247±5 vs 155±5%; p<0.05). Summary/Conclusions. The data show that LMWH nadroparin improved the patients’ hypercoagulable state, as demonstrated by the reduction of plasma TAT levels, but did not influence the endothelial perturbation during chemotherapy, as measured by the levels of endothelial activation markers (i.e. PAI-1 and vWF). The alterations of endothelial markers may be possible candidates to identify ambulatory cancer patients at high thrombotic risk during chemotherapy.

1715
CHANGES IN PLASMA HEMOSTATIC VARIABLES IN AMBULATORY CANCER PATIENTS ENROLLED IN A TRIAL OF THROMBOPROPHYLAXIS WITH LOW-MOLECULAR-WEIGHT HEPARIN (LMWH) NADROPARIN DURING CHEMOTHERAPY (PROTECHT)

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Background. The results of the PROTECHT clinical trial (Agnelli et al., Lancet Oncology 2009) demonstrated the efficacy of thromboprophylaxis with the LMWH nadroparin in reducing the rate of thromboembolic events in ambulatory patients receiving chemotherapy for metastatic or locally advanced solid tumors. Aim. In this placebo-controlled randomized clinical trial, a study of plasma thrombotic markers [i.e. thrombin-antithrombin (TAT) complex, plasminogen activator inhibitor-1 (PAI-1)] and von Willebrand factor (vWF)] was planned to evaluate: 1. the biomarkers’ predictive value for thrombosis, and 2. the modulation of the biomarkers by thromboprophylaxis during chemotherapy. Methods. Plasma samples from 141 patients were available for analyses at baseline (T0), i.e. before starting treatments (96 LMWH/45 placebo). Additional plasma samples from 31 study subjects (17 LMWH/14 placebo) were also obtained: before the 3rd chemotherapy cycle (T1), and at the end of treatments (T2). One hundred healthy subjects acted as controls. Results. At T0, the levels of TAT, PAI-1 and vWF were significantly increased in patients compared to healthy controls (p<0.05). In the 31 patients analyzed over time, a significant reduction of TAT levels, from T0 to T1, occurred in subjects given LMWH, but not in those receiving placebo (p<0.05). After discontinuation of the study drug (T2), TAT levels returned to pre-treatment values. PAI-1 levels remained steadily elevated from T0 to T1 to T2 in both LMWH and placebo groups, while vWF levels were even significantly increased (p<0.05) over the chemotherapy period, with a similar profile in both study arms. Three out of the 141 patients experienced thrombosis during treatment. The small number of events did not allow to calculate the predictive value of plasma markers, but, interestingly, patients with thrombosis had significantly higher baseline levels of vWF than those without (247±5 vs 155±5%; p<0.05). Summary/Conclusions. The data show that LMWH nadroparin improved the patients’ hypercoagulable state, as demonstrated by the reduction of plasma TAT levels, but did not influence the endothelial perturbation during chemotherapy, as measured by the levels of endothelial activation markers (i.e. PAI-1 and vWF). The alterations of endothelial markers may be possible candidates to identify ambulatory cancer patients at high thrombotic risk during chemotherapy.

1716
ALTERATION IN HUMAN ENDOTHELIUM CELL FUNCTION INDUCED BY HYPERGLYCEMIA AND HYPERLIPIDEMIA

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Excess vascular permeability due to secondary factors could promote a more aggressive development of hematologic malignancies. We studied a development of endothelium dysfunction by hyperglycemia or hyperglycemia employing cultivating primary human endothelium cells (HUVEC) in different conditions. In order to study the effects of hyperglycemia we assessed cell response to normal glucose (5.6 mM) or high glucose (25.0 mM) content and used high sorbitol content (glucose 5.6 mM + sorbitol 19.4 mM) as osmolarity control. For hyperlipidemia, we used postprandial plasma collected from healthy volunteers 2.5 hrs following a fatty meal (300 g bread, 60 g butter); fasting plasma samples isolated from the same healthy volunteers served control. We than cultured HUVEC in a presence of 10% of plasma with high lipid content (HL) or high lipid level (NL) for 6 days. Lipid analysis revealed higher amount of triglycerides in postprandial plasma and similar level of high density and low density lipoproteins and cholesterol probably due to presence of excess amount of cholesterins and remnant lipid-containing particles. Staining cells with lipid-soluble dye (Oil-red O) reveal accumulation in cellular cytoplasm lipid-containing granules after two days of incubation. Growth rate analysis reveal that incubation with HL strongly suppresses cell proliferation and decreases percentage of viable cells, even more than high glucose level. We have observed that

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when cultured in high glucose or high fat conditions for 2 days, HUVEC cells presented considerable signs of senescence, as detected with α-galactosidase activity assay. We further found that a combined exposure to high lipid and high glucose has a substantial synergistic effect on induction of endothelial cells senescence. That could cause inability of the cells to sustain proper vascular homeostasis. Constant activation of pro-proliferative signaling pathways could result in decrease in cell viability by compensatory mechanisms, inhibiting excessive signal. We revealed that protein kinase Akt1 is strongly activated by HL in shot (30min) or long (6 days) incubation. Cells could be unable to proper react to growth factors stimulation and therefore decrease viability and become unable to sustain a barrier function. We also analyzed cellular contacts by staining for intercellular junctions (VE-cadherin and PECAM) and found that cells incubated in presence of high glucose and high lipid conditions in confluent monolayer were unable to sustain tight cell-cell contacts, possibly due to decreased level of particular proteins. In summary, we found that chronic exposure of endothelial cells to high glucose and high lipid conditions could alter cell viability and decreases barrier function.

1717
D-DIMERS: A SIMPLE, SENSITIVE MARKER OF PROCOAGULANT ACTIVITY
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Background. It is established that occurrence of thrombosis requires the coexistence of two or more, acquired or congenital, thrombophilic factors. Acquired thrombophilic factors can be easily recognized. Congenital thrombophilic factors are difficult to be detected, because of biochemical and practical issues; a thorough investigation is very expensive, while only half of all congenital defects are known. Aims. The aim of the current study was to evaluate the measurement of D-Dimers as a simple and sensitive screening test, which could reveal early activation of coagulation, thus leading to investigation of congenital thrombophilic factors and/or adoption of preventive measures. Subjects-Methods. Our study group consisted of 32 individuals, with no personal history of thrombosis, who were tested for thrombophilia. We used ACL Advance coagulation analyzer in order to measure plasma total antithrombin III (ATIII), protein C (PC), protein S (PS), factor VIII (FVIII) and factor FVII (FVII) activities. We used the same analyzer in order to measure plasma total homocysteine and to investigate the existence of V-Leiden mutation. We performed by use of an in house PCR protocol. Finally, ACL Advance coagulation analyzer was used for a latex-based determination of plasma total fibrin D-Dimers. Demographic data and medical history of participants were collected through a structured questionnaire. Results: Study group consisted of 32 individuals, with no personal history of thrombosis at a relatively young age. Two of them also reported heavy consumption of cigarettes and coffee. Two out of the three Group B subjects with positive D-Dimers had sickle/beta-thalassemia or sickle cell trait, respectively, while the third subject reported heavy cigarette and coffee consumption.

Conclusions: D-Dimers’ test may be a simple and sensitive marker of early coagulation activity. The slightly elevated D-Dimers’ levels in all subjects with positive D-Dimers, suggest that laboratories must use D-Dimers’ tests with high sensitivity, not only to exclude a thromboembolic episode but also to indicate the presence of hypercoagulability.

1718
STUDY OF URINARY 11-DEHYDROTROMBOXANE B2 AS A MARKER OF HYPERCOAGULABLE STATE IN CHILDHOOD HEMATOLOGICAL MALIGNANCIES
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Background. CXancer can confer a hypercoagulable state through an altered balance between the coagulation and fibrinolytic systems, which can be related to long term treatment. Association of prothrombotic markers with thrombosis in pediatric oncology is conflicting. Patients who exhibit elevated 11 dTXB2 levels, as a marker of hypercoagulability; may be at increased risk for thrombembolic events. Aims: The aim of the work is to predict thromboembolic state in childhood hematological malignancies by estimation of 11-dehydrothromboxane B2. Subjects and methods. This study was conducted on twenty-five patients with newly diagnosed hematological malignancies. They were followed up and treated in Oncology unit, Pediatric department, Zagazig University Hospitals. Patients were subjected to full history taking and thorough clinical examination with special attention to thromboembolic manifestations. Laboratory investigations were done before and after four months from starting chemotherapy: CBC, PT, PTT, urine creatinine, D-dimer and estimation of urinary 11 dehydro-thromboxane B2 (TXB2) level. Results. Our data showed that there was no significant clinical presentations suggesting presence of thrombosis either before or after chemotherapy in both leukemic and lymphoma patients. There was a significant increase in D dimer and TXB2 / creatinine ratio levels in leukemic and lymphoma patients before the start of chemotherapy when compared to the controls. Also, there was a highly significant decrease in these levels after 4 months from starting chemotherapy in leukemic and lymphoma patients. Our data showed that there was significant correlation between TXB2 / Creatinin ratio values and D-dimer values, while, there was no statistical correlation between TXB2 / Creatinine ratio values before and after 4 months from chemotherapy in both leukemic and lymphoma patients. Conclusions. children with hematological malignancy are at high risk of thrombosis; even if asymptomatic. So, those patients should be screened for additional prothrombotic risk factors and appropriate measures should be taken to prevent the development of thrombosis.

1719
IMPLEMENTATION VALIDATION OF THE REAL-TIME PCR FOR FACTOR V LEIDEN AND PROTHROMBIN G20210A MUTATION ON THE GENEXPERT DX SYSTEM
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Background. Venous thromboembolism (VTE), including deep venous thrombosis and pulmonary embolism, has an estimated annual incidence of approximately 100 per 100,000 in the general population and is a major source of morbidity and mortality (White, 2003). Factor V Leiden and the prothrombin mutation are the most common inherited risk factors for VTE. After diagnosis of VTE, these thrombophilic risk factors are frequently determined to etiologically performed for etiologic purposes and risk assessment of recurrent thrombosis. Aim To validate the Xpert HemosIL FII & FV kit (Instrumentational Laboratory, IL), a commercially available (CE-IVD and FDA approved) kit on the GeneXpert Dx System (Cepheid), for detection of factor V Leiden and prothrombin G20210A mutation. Methods All validation experiments were performed with 50 µl of EDTA whole blood. Molecular testing was performed on the GeneXpert Dx System according to the manufacturers instructions. This system combines extraction, real-time PCR, detection and result interpretation. The assay was checked for analytical sensitivity, specificity, precision and accuracy following international publications on molecular validation methods (e.g. ACMG, 2006). Twenty two patient samples were included in Immeld Hospital over a 4-month period to evaluate the clinical performance, these samples were tested with the HemosIL FII & FV kit as well as with the Light Cycler FRET system (Roche), performed in the University Hospitals Leuven. Results Sensitivity: The cycle
threshold (Ct) values for the prothrombin and factor V Leiden mutation ranged from 21.2 to 25.6 Ct and 21.8 to 28.1 Ct respectively. In this Ct-area errors related to limited or excess sample are rare. According to the manufacturer a volume of 50 µl of EDTA whole blood is sufficient to obtain a correct genotype. Specificity: Silent mutations (SNPs) in the probe binding area can occur. The manufacturer demonstrated that known SNPs like A1691G, G1626A, G1629A can lead to an invalid result. However, valid results always revealed a correct genotype. Precision: Two samples (Homozygous normal - FV homozygous normal patient sample and FII homozygous normal - FV heterozygous mutant control sample), were analyzed in triplicate on 3 different days. The standard deviation (SD) (Ct) was between 0.5 and 0.8 for both samples, meeting our validation criteria of SD < 1 Ct. Accuracy: The 2010 INSTAND controls (whole blood), the NIBSC WHO reference panels and a CE-IVD / FDA FII & FV control (IL) were used to check the accuracy of the HemosIL kit. A 100 percent agreement was found. Clinical performance: In 3 of the 22 patients APC resistance was detected and subsequently a heterozygous Factor V Leiden mutation was genetically confirmed. Nineteen patients were tested for the prothrombin mutation and only one heterozygous mutation was found. The other patients were homozygous normal for both factors. A 100 percent agreement with the Light Cycler FRET method was observed. Conclusion: The HemosIL FII & FV assay on GeneXpert Dx System met all our analytical and clinical validation criteria and was implemented in the daily routine.

1720
THROMBOPHILIA AND CRYPTOGENIC FOCAL EPILEPSY AMONG CHILDREN AND YOUNG ADULTS
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Background. Epilepsy has frequently a multifactorial aetiology and recognizes a single cause is often impossible. Advanced neurodiagnostic investigations resulted in decreased rate of epilepsy of unknown etiology. Magnetic Resonance Imaging (MRI) brain allowed to identify the cause of focal epilepsy and generalized epilepsy, respectively in 70% and 50% of the cases. To determine the pathogenesis of idiopathic focal epilepsy in MRI negative cases in children and young adults, this study would investigate a possible role of prothrombotic risk factors. Methods. We studied 48 patients (32M, 16F), aged between 1 and 32 years (median age 11.5 years). All were affected by epilepsy in which it was possible to investigate a possible role of prothrombotic risk factors. Eighty heterozygous and eight homozygous patients presented increased plasmatic and urinary levels of homocysteine. Forty-four of these sixty (73%) patients presented normal homocysteinemia, thiry-six (60%) presented with MTHFR heterozygous genotype and seven (16%) with MTHFR homozygous genotype. Sixteen of sixty (27%) patients presented elevated levels of homocysteine, eight (13%) with MTHFR heterozygous genotype and eight (15%) with MTHFR homozygous genotype. Twenty-six were treated with low molecular weight heparin (enoxaparine 100 U/kg s.c. x 2 daily), then they all continued the treatment with oral anticoagulant for 6-12 months. Five patients were treated with antiaggregation therapy and all of them performed long term prophylaxis with folic acid and B group vitamins. About remaining patients, since venous thrombosis was subsequent of thromboembolic event, three patients did not receive treatments. Conclusions. Our data raise the question whether MTHFR gene polymorphism alone, with or without hyperhomocysteinemia, may somehow contribute to thrombophilia and suggest to perform anticoagulant prophylaxis in all patients with MTHFR mutation with previous thrombotic events in case of other thrombotic risk factors (pregnancy, oral contraceptives, sepsis and immobilization). Not always the MTHFR polymorphism leads hyperhomocysteinemia. This may be due to the effect of homocysteine levels on thrombotic disease and to the variations of homocysteinemia that may depend on others conditions. The impact of the MTHFR mutation as independent thrombophilic marker on outcome and recurrence risk of venous thrombosis needs to be further investigated.

1721
HEMOSTATIC AND RHEOLOGIC CHANGES OF ACUTE MYOCARDIAL INFARCTIONS IN NIGERIANS
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Background. Myocardial infarction (MI) is defined as necrosis of a portion of cardiac muscle caused by obstruction in coronary artery through either arteriosclerosis, a thrombus or spasm. The causative factors has been well documented and its risk has been reduced to the barest minimum in advanced countries of the world while in developing countries such as Nigeria, the advent of MI as a major cardiovascular problem is moderately recent. Therefore, researches into the responses of rheologic and fibrinolytic parameters are modestly new and ongoing. Aims. We therefore aimed to highlight basic information on haemorheologic and fibrinolytic parameters with a view to indicate their possible use as diagnostic and prognostic indices in MI. Methods: We investigated longitudinally, 10 acute myocardial infarction (AMI) patients together with 20 age and sex -matched apparently healthy subjects as controls. Blood samples were taken at the point of admission (Day 0), on the 4th and 7th day respectively after treatment has commenced. Rheologic and fibrinolytic indices such as complete blood count (CBC), erythrocyte sedimentation rate (ESR), Plasma Fibrinogen concentration (PFC), D-dimer concentration (DDC), Euglobulin lysis time test (ELT) and platelet aggregation (PA) were performed in this study. Results: We recorded a significantly reduced values of haematocrit and fibrinolytic activity coupled with significantly increased D-dimer levels, PFC, ESR and IV in AMI patients on admission compared with controls (P<0.05). However, PFC, DDC and ELT became significantly lowered from the 4th day of admission while all the parame-
Inflammatory biomarkers in acute coronary syndromes

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Background. Acute coronary syndromes occur in response to inflammation, plaque rupture and subsequent thrombosis. Inflammation is associated with the progress and stability of atherosclerotic vascular lesions and much interest has focused in some inflammatory parameters, such as white blood cell (WBC) count, interleukin-6 (IL-6) and matrix metalloproteinases (MMPs). Traditionally elevated WBC count considered as an indicator of systemic inflammation, but it has also been accepted as part of the healing response following acute myocardial infarction. IL-6 is an inflammatory cytokine which appears to play an important role in atherogenesis. Many studies have demonstrated that MMP-mediated degradation of extracellular matrix is critical for atherosclerotic plaque rupture, responsible for acute atherothrombotic syndromes, with acute coronary syndromes. Methods: We studied 45 patients (38 males - 12 females, mean age 64.4±12.9 years), either with ST-segment elevation (STEMI) or without ST-segment elevation (non-STEMI) myocardial infarction. From blood samples, which were taken from a peripheral vein, in the first post-infarction day, human pro-MMP-10 and IL-6 plasma levels were measured, using an enzyme immunoassay method. WBC count was also measured in the same samples. Results: WBC (and neutrophils), MMPs and IL-6 levels were found elevated to our study population. No statistically significant differences between STEMI and non-STEMI could be demonstrated. Results are summarized in the table. Conclusions. All inflammatory parameters were found elevated in patients with acute coronary syndromes, possibly because of the existing inflammatory process. Whether elevated WBC count, MMPs or IL-6, are only a marker of the inflammatory process or a direct risk factor for acute coronary events remains unclear.

1723
D-DIMER ASSAY IN EGYPTIAN PATIENTS WITH GAUCHER DISEASE: CORRELATION WITH BONE AND LUNG INVOLVEMENT

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Background. Gaucher disease (GD) is the most frequent lysosomal storage disorder. Bone and lung involvement are two major causes of morbidity in this disease. D-dimer is a reliable indicator of active microvascular thrombosis, even in patients without overt hypercoagulation. Aim: This study aimed to assess D-dimer levels in GD correlating this marker to clinical characteristics and radiological parameters to investigate its role as a potential predictor for the occurrence and severity of skeletal and pulmonary manifestations. Methods: The study population consisted of 56 Egyptian patients with GD; 36 had type 1 (64.3%) and 20 had type 3 (35.7%). Thirty healthy individuals were enrolled as a controls. All patients were receiving regular ERT (the recombinant glucocerebrosidase enzyme). Laboratory investigations were done including complete blood count, liver function and quantitative plasma D-dimer assay. Radiological; investigations X-rays long bones, abdominal ultrasound for assessment of liver and splenic volume, high resolution CT chest (HRCT) as well as magnetic resonance imaging for pelvic spines and femur bones. Results. All Gaucher patients had shown remarkable improvement of growth and hematological parameters, as well as reduction of hepatic and splenic volumes after 6 months of the ERT. D-dimers in all Gaucher patients were significantly higher compared to controls with a mean of (2011±499 and 396±94 ng/ml respectively, P=0.03). D-dimers were significantly higher in type III compared to type I patients with a mean of (1063.5±414 and 830±275.2 ng/ml respectively, P=0.05). Pulmonary involvement and HRCT findings (ground glass appearance, interlobular thickening) were present in a higher percentage among type III compared to type I (71%, 37.5% respectively) (P=0.05). While bone involvement and MRI findings (bone marrow expansion, cortical thinning) were present in higher percentage of type III compared to type I patients (24%, 21% respectively) (P=0.65).

1725 DETECTION OF THROMBOPHILIC MUTATIONS IN WOMEN WITH ADVERSE PREGNANCY OUTCOMES

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Introduction. Inherited thrombophilia is associated with a high risk of pregnancy complications (including early recurrent miscarriage, intrauterine fetal death, intrauterine growth restriction (IUGR) and preeclampsia. Factor V Leiden (FVL) G1691A, methylenetetrahydrofolate reductase (MTHFR) C677T/ A1298C, and factor II (FII) G20210A mutations are important causes of thrombophilia. The aim of this study is to evaluate the prevalence of these mutations in a group of obstetric patients. Materials and Methods. The study enrolled 55 women with obstetric complications divided in: maternal complications - 3 patients with post-section venous thromboembolism, and fetal complications - 31 women with 1-2 early miscarriages, 15 with >5 early miscarriages, 6 with IUGR, 2 with preeclampsia and 4 with intrauterine fetal loss. DNA was isolated from blood samples using Wizard Genomic DNA Purification Kit (Promega) and melting curve analysis (LightCycler 480 platform) for Factor V Leiden G1691A and FII G20210A mutation. The presence of C677T and A1298C mutations was investigated using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism method based on Hinf I and Mbo II endonuclease digestion. Results. FV Leiden was detected in 17 women (29.3 %), 3 were homozygous and developed VTE. Five women (9.06%) had heterozygous prothrombin mutation and three of them presented intrauterine fetal death with severe IUGR. A high frequency of MTHFR mutations was obtained: 41 women (70.6%) with C677T mutation (9 homozygous, 32 heterozygous) and 28 women (48.2%) with A1298C mutation (6 homozygous, 22 heterozygous). Three patients had 3 simultaneous mutations, 29 had 2 mutations and 3 patients presented 1 mutation. A high frequency of MTHFR mutations was found in women with obstetric complications divided in: maternal complications - 3 patients with post-section venous thromboembolism, and fetal complications - 31 women with 1-2 early miscarriages, 15 with >5 early miscarriages, 6 with IUGR, 2 with preeclampsia and 4 with intrauterine fetal loss. DNA was isolated from blood samples using Wizard Genomic DNA Purification Kit (Promega) and melting curve analysis (LightCycler 480 platform) for Factor V Leiden G1691A and FII G20210A mutation. The presence of C677T and A1298C mutations was investigated using Polymerase Chain Reaction - Restriction Fragment Length Polymorphism method based on Hinf I and Mbo II endonuclease digestion. Results. FV Leiden was detected in 17 women (29.3 %), 3 were homozygous and developed VTE. Five women (9.06%) had heterozygous prothrombin mutation and three of them presented intrauterine fetal death with severe IUGR. A high frequency of MTHFR mutations was obtained: 41 women (70.6%) with C677T mutation (9 homozygous, 32 heterozygous) and 28 women (48.2%) with A1298C mutation (6 homozygous, 22 heterozygous). Three patients had 3 simultaneous mutations, 29 had 2 mutations and 3 patients presented 1 mutation. 3 Dept of Obstetrics and Gynecology, Emergency University Hospital, Bucharest, Romania

16th Congress of the European Hematology Association
with the highest morbidity. Thrombophilic molecular evaluation should be performed in selected women with adverse pregnancy outcomes. Funding. This work was supported by the grant PN 42-099 from the Romanian Ministry of Research and Technology.

1726
RENAral INFARCTION: CASE REPORTS IN PATIENTS WITH THROMBOEMBOLIC RISK FACTORS
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Background. The renal infarction is a rare, but serious and often misdiagnosed condition. There is not a therapeutic consensus to treat this disease, because of the frequent episodes reported in different series. We can find different treatment choices: surgery, fibrinolysis and anticoagulation. Aims. Review of the cases of renal infarction diagnosed in our hospital: clinical diagnostic, therapeutic management and evolution. Case 1: 52-year-old male, with history of hypertension, active smoker, pulmonary emphysema, generalized atheromatous, chronic vascular ischemia, angor haemodynamic, cocaine use in treatment with methadone, derived from another hospital due to acute and refractory to analgesic pain in right hypochondrium, radiated to epigastric and neck associated with nausea and vomiting. Abdominal exam: painful to the palpation in flank and right iliac fossa, without signs of peritoneal irritation. Ultrasound: normal size and structure kidneys. Angio-CT: atheromatous stenosis of the principal branch of the right renal arterial. Reevaluation CT: right kidney with decrease of size and poor parenchymal opacification, suggesting chronic renal infarction. Treatment: conservative with oral anticoagulation developing good response. Angio-CT: resolution of the injury with arterial permeabilization. Case 2: 65-year-old male with history of hypertension, presented right hypochondrium pain, persistent and refractory to analgesic treatment. Abdominal exam: painful to the palpation in right flank, without signs of peritoneal irritation. Angio-CT: extensive renal infarction involving the middle third of the right kidney, with hypoperfusion in this area. Treatment: Fibinolysis and non fractionated heparin. Hipercagulability study revealed positive lupus anticoagulant suggesting an antiphospholipid syndrome. Genetic study showed heterozygote of the MTHFR with high levels of homocisteine. Case 3: 84-year-old male with history of hypertension, cardiac insuficiency, auricular fibrillation and chronic pulmonary disease, came to emergency from another hospital due to abdominal pain for 11 days, with nausea and vomiting, suggestive of cholecystitis. Ultrasound: bilial bladder with inflammatory signs, cortical cysts in right kidney. CT: severe aortollic atheromatous, hypoperfusion renal area, 35% right and 15% left. Patient was in surgery because of perforated cholecystitis, then continued with anticoagulation. Echocardiogram: Normal.

Case 4: 56-year-old man with history of myocardial infarction, prostate cancer and hypertension, presented abdominal pain and constipation for 15 days, associated to weight lost. Abdominal exam: reduced bowel sounds, painful to the palpation in right flank, without signs of peritoneal irritation. Angio-CT: stenosis of transverse colon because of tumor presence, splenic and bilateral renal infarction. At the same time it is detected positive for lupus anticoagulant. During hospitalization patient developed a small cerebral infarction, kidney failure, and worsening of previous clinic described. He was under surgery because of intestinal obstruction, subsequently worsening of kidney failure, multiorgan failure and death. Comments. Although acute renal infarction is a rare entity, it is necessary to suspect it in patients with abdominal pain and risk factors for thromboembolism. Early diagnosis is critical in order to diminish the morbimortality of this disease. Physicians must extend the ethiology study, because there are many entities that can cause this pathology in patients without known risk factors like use of cocaine, sepsis, neoplasm and thrombophilia.

1727
EVALUATION OF THE EFFECT OF SUBCLINICAL HYPOTHYROIDISM ON BLOOD COAGULATION PARAMETERS
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Background. Subclinical hypothyroidism (SCH) is defined as a status of mildly elevated thyrotropin (TSH) levels associated with normal total or free T3 and T4 values. In general, it is an asymptomatic entity, usually diagnosed after routine screening or while exploring nonspecific clinical presentations, which has been associated with enhanced cardiovascular risk. There has been considerable controversy regarding the alterations of blood coagulation parameters in patients with SCH as both hypercoagulable and hypocoagulable states have been referred. Aims. To evaluate platelet activation and coagulation disorders in patients with SCH and the influence of TSH, peripheral thyroid hormones (T3, T4) and thyroid autoantibodies such as thyroid antithyroglobulin (anti-TG) and antithyropheroxidase (anti-TPO) on them. Methods. A total of 24 patients with SCH (males/females: 10/14) and 24 euthyroid control subjects matched for age and gender were enrolled to the study. Platelet indices (platelet count (PLT), mean platelet volume (MPV) and platelet distribution width (PDW)) were measured in whole blood samples by flow cytometry method with the use of XE-5000 Sysmex (ROCHE). Coagulation parameters (prothrombin time (PT), activated partial thromboplastin time (aPTT), international normalized ratio (INR), protein C and protein S) were measured in plasma samples using a fully automated analyser (ACL Top, ALAPIS). Serum TSH, FT3, FT4, anti-TG and anti-TPO levels were determined by electrochemiluminescence immunoassay with Elecsys Modular E170 analyser (ROCHE).

Results: The results are presented in Table 1. The levels of MPV and PDW were significantly higher in the SCH group than in the euthyroidic one (p<0,05). No differences were observed in other coagulation parameters between SCH patients and control subjects. The above indices were not found to be correlated neither with the thyroid hormone concentrations nor with the measured autoantibodies levels. Summary/conclusions: Subclinical hypothyroidic patients present alterations in some blood coagulation parameters, which are indicative of platelet activation. The speculation that elevated MPV values may be involved in the increased risk of atherothrombotic complications in subclinical hypothyroidism should be further investigated.

Table 1: Platelets with SCH and normal controls.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with SCH</th>
<th>Normal controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLT (K/UL)</td>
<td>277 ± 108.6</td>
<td>278 ± 96.0</td>
<td>0.82</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>9.4 ± 0.9</td>
<td>9.3 ± 0.8</td>
<td>0.003</td>
</tr>
<tr>
<td>PDW (%)</td>
<td>1.9 ± 0.2</td>
<td>1.8 ± 0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

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ANTICARDIOLIPID ANTIBODIES IN THE REAL WORLD: WHAT IS THE IMPACT OF A CONFIRMATORY TEST AT 12 WEEKS?

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Background. Because of the possible transient nature of antiphospholipid antibodies, Sydney classification criteria for antiphospholipid syndrome recommend a confirmatory test, which was postponed at least 12 weeks apart from the first measurement. Aims. The aim is to evaluate the impact of this confirmatory test in patients referred to our hospital and having a first positive anticardiolipin antibody (aCL) ELISA. Methods. Home made aCL ELISA (IgG and IgM) was performed according to the requirements of the European Forum on antiphospholipid antibodies using cardiolipin for coating and with locally established cut-off values (≥99th percentile 15UGPL -7UMPL). CV was 10%. Results. Among the patients prospectively included in our database (2005-2010), we identified 91 patients with a positive aCL ELISA either IgG (57) or IgM (41) or both (13). Median and range values were 60.9 (15-576) UGPL and 17 (7-288) UMPL for IgG and IgM respectively. Among them, 53 had a positive second result. Patients persistently positive had a higher level of antibodies at the first test: median and range was 63.8 (15-576) and 17 (7-288) UGPL for persistent and non persistent aCL IgG and IgM respectively. 8.3 (7-14) UMPL for persistent and non persistent aCL IgM (borderline statistical. Conclusions. At or beyond 12 weeks 58% were found to remain positive, but a subset of patients with further testing could become negative. Thus a second, confirmatory test, even when performed at 12 weeks rather than at 6 weeks as previously recommended, could be insufficient. The clinical relevance of the differing patterns of persistency remains to be elucidated.

PROTHROMBIN MUTATION G20210A AND MUTATION MTHFR - RISK FACTOR FOR RECURRENT FETAL LOSS

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Background. In the recent years, thrombophilia as a risk factor for pregnancy complications and fetal loss gained much attention in the scientific community. However, data on this topic in the literature are conflicting. Prothrombin mutation G20210A and mutation MTHFR result in an increased susceptibility to develop venous thrombosis. The association of clinical manifestations can be heterogeneous as regards severity as well as type of event (VTE or obstetric complication). Aims. This study estimates the risk factor of the presence of inherited thrombophilia genetic defects in women with recurrent fetal loss. Methods. 95 women with a history of recurrent fetal loss were referred to our laboratory for thrombophilia genetic testing (prothrombin G20210A, MTHFR 677C>T) by PC-RFLP (trans-novel, during one year (2009). Results. 88 (92.6%) women had a positive genetic testing: 24 (27.27%) had prothrombin G20210A mutation, 4 (4.54%) had homozygous for MTHFR, 29 (32.95%) had heterozygous and 31% (35.22%) had double heterozygosity. Conclusions. Considering the higher percentage of these genetic mutations in women with multiple fetal losses, there are many voices in the scientific community who suggest that maternal screening before pregnancy is a must, in spite of the high cost of this investigation.

INHERITED THROMBOPHILIA IN WOMEN WITH RECURRENT PREGNANCY LOSS

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Background. Recurrent pregnancy loss (RPL) is a common clinical problem. Several groups have reported an association between inherited thrombophilia and RPL. Aims. The aim of this study was to investigate the prevalence of inherited thrombophilia in women between the ages of 18 and 45 referred to our clinic for evaluation of thrombophilia as the etiology of RPL. Methods. We retrospectively analyzed the records of women who had complete thrombophilia testing for RPL, defined as 2 or more otherwise unexplained pregnancy losses, between the period of January 2008 and December 2010. The following thrombophilic disorders were included: presence of protein C deficiency, protein S deficiency (PS), anti-thrombin deficiency (AT), activated protein C resistance (APCR), factor V G1691A (FVL), prothrombin G20210A (PTG) and Methyl tetrahydrofolate reductase C677T (MTHFR) done outside of pregnancy or the immediate post-partum period. Results: A total of 43 patients with complete data were identified. Median age was 33 years (range: 20-45). Median number of pregnancy losses was 3 (range: 2-5). 15 women (55%) were found to have a thrombophilic condition. PS deficiency was identified in 1 woman (2%), AT deficiency in 1 (2%) and APCR in 2 (4%), while no cases of PC deficiency were identified. In addition, 2 (4%) had FVL (same women identified as having APCR, both heterozygous), 1 (2%) had PTG and 11 (25%) had MTHFR (10 heterozygous and 1 homozygous). Summary/conclusions. Inherited thrombophilias are not uncommon in women with RPL. These results suggest that testing for thrombophilia may be warranted in women with RPL. A prospective study with a control group and more detailed thrombophilia testing, including confirmatory DNA analysis, to assess the true prevalence and significance of such defects in women with RPL is highly warranted.
been identified as an independent risk factor of cardiovascular disease and thromboembolic incidents. Aims: The purpose of our study was the evaluation of IPF, MPV, PDW and levels of homocysteine (Hcy) in patients with cardiovascular disease. Methods: Our material consisted of 70 patients, (37 males and 33 females, aged 18-74 years old) suffering of coronary artery disease. A second group consisted of 40 healthy persons (20 males and 20 females matched for age and ethnicity). IPF, MPV and PDW were measured by flow cytometry with the SYSMEX XE2100 analyser. The levels of Hcy were determined with immunofluoropolarimetry on the AxSYM-plus analyser. Exclusion criteria for entry into the study was the presence of co-existing thrombotic or hematomatological disease. Results: Patients with cardiovascular disease presented significantly higher IPF, MPV, PDW and Hcy values than normal controls (all p<0.05). A positive correlation was found between the values of IPF and Hcy (r=0.648, p<0.01) while no positive correlation was found between the value of Hcy and the value of MPV or PDW. Conclusions. IPF, MPV, PDW and Hcy are elevated in patients with cardiovascular disease. From our study is concluded that the combination of measurement of IPF, MPV, PDW and homocysteine is useful in patients suffering with cardiovascular disease as indices of risk of new cardiovascular incidents.

**1733**

**HEMATURIA AND GINGIVAL BLEEDING IN A PREGNANT WOMAN**

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**Background.** Antagonists of vitamin K (Vit K) are currently the most used rodenticides. Ingestion causes nausea, vomiting and bleeding within 36-48 hours. Serious intoxication is caused by repeated doses or by a single massive dose (>15 g). Diagnosis is based on clinical findings and the determination of the specific toxic is interesting from a forensic point of view. Bleeding risk may last for several weeks for compounds of long average life and Vit K treatment may be necessary for weeks or months until total recovery of coagulation parameters. Case report: 17 weeks pregnant woman without pathological history, was admitted for mild bleeding (hematuria and gingival bleeding) for 1 week. Laboratory findings presented global changes in coagulation parameters. Previous studies are normal. Amniocentesis had been performed 3 weeks before without complications. Analytical findings are shown in Table 1. A moderate to severe acquired Vit K dependent clotting factors deficiency was confirmed: FII 3.3%, FV 99.9%, FVII 3.1%, FVIII 134.7%, FIX 8.8%, FX <5%, FXI 78.7%, FXII 94.4%. After the initial intravenous Vit K treatment bleeding improved and clotting parameters normalized. 21-48 hours oral Vit K was initiated developing recurrence of coagulation disorders, suspecting the possible action of some long life substance. Differential diagnosis- bowel disease, malabsorptive, drugs ingestion, oral anticoagulants and/or rodenticides were discarded. The patient reported the use of rodenticide (brodifacoum) last year due to a rat invasion at her home, but denies current contact. Given the high suspicion of possible intoxication by superwarfarins, determination of levels in blood and urine samples were studied resulting positive in both samples. Hemo-stasis study was conducted in cohabitants to discard multiple intoxication resulting normal.

The patient was discharged from hospital with treatment of oral Vit K (initial dose 40 mg/day) lasting for 2 months and pregnancy came to term without complications. Conclusions: The ‘superwarfarins’ displays exert its toxic effect by inhibiting the reduction of Vit K, preventing its activation and resulting in a lengthening of TP, and in more serious cases lengthening of aPTT. Intoxication should be suspected when sustained and high doses of intravenous Vit K are needed to correct coagulation parameters, and this treatment may be sustained for one or two months. The most common cause is the accidental ingestion, especially in children, although the manifold intake in familiar surroundings must be discarded. Other causes are important due to legal involvement.

**1734**

**IS PSYCHOLOGICAL ADJUSTMENT TO A DIAGNOSIS OF ACUTE LEUKAEMIA INFLUENCED BY ACCEPTANCE OF DIAGNOSIS AND AMOUNT OF TIME ELAPSED TO ACCEPTANCE?**

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**Background.** Coping with cancer is a subject intensely analyzed by psycho-oncologists. It was proven that there are several factors that help patients to develop an effective coping: personality traits, efficient coping with other problems, internal and external psychological resources. Aims: We intend to analyze how acceptance of the diagnosis and the amount of time elapsed to acceptance influence coping with acute leukemia. Methods: From November 2007 to April 2009 we conducted 100 interviews with patients admitted at the Cluj Hematology Clinic, all with a diagnosis of acute leukemia, and treated with standard protocol chemotherapy. Patients were asked how long did it take to accept their diagnosis and to rate the degree of acceptance on a scale from 1 to 10 (1 = does not accept diagnosis, 10 = total acceptance). At the end of the interview (which focused on more aspects than those addressed by this study), the author (a psycho-social counselor and clinical hematologist) determined patients’ coping strategy to leukemia as either efficient or inefficient. Data was analyzed with SPSS and Excel. Correlations between time/degree of acceptance of diagnosis and coping were made using Cramer contingency coefficients. Patients signed informed consent, and the study was approved by the Ethics Committee of Medicine and Pharmacy University Cluj-Napoca, Romania. Results: Patterns of efficient coping identified in this group of patients were: fighting spirit, realistic approach toward disease, direct confrontation of disease, overcoming denial, and considering every possible disease outcome (favorable or unfavourable). In this group, 93% of the patients developed an efficient coping to acute leukemia Patterns of inefficient coping were: long-term denial of disease or prognosis, passive attitude, blaming others, anger, depression, and concealment of truth. The time period to accept the diagnosis ranged from immediate to four months. During the interviews patients were asked to describe and analyze the reason for their immediate or delayed acceptance of diagnosis. The reasons for the immediate acceptance were: presence of severe or neoplastic disease in patient’s or his family’s medical history, severe symptoms at initial meeting, hearing of suspicion of neoplastic disease from a different hospital, belief in a cure, hearing the diagnosis after a prolonged investigations. The degrees of acceptance varied from 4 to 10 on the scale described above, where about 2/3 of the patients ranked at ≥8. The time period to acceptance of diagnosis was not statistically relevant associated with efficient coping (F=8.75, p=0.006>0.05). A high degree of acceptance of the diagnosis was significantly associated with efficient coping (F=7.13, p=0.019<0.05). Conclusions: A high degree of acceptance of diagnosis influences the development of an efficient coping strategy. The time period to acceptance of diagnosis does not appear to influence coping. Patients with a low degree of diagnosis acceptance (4-6 on the 1-10 scale above) developed an inefficient coping and these patients would benefit from targeted and individualized psychological support.

**1735**

**QUALITY OF LIFE IN NON-HODGKIN’S LYMPHOMA PATIENTS TREATED WITH 21 R-CHOP IN ASSOCIATION WITH PROFILACTIC GRANULOCYTE GROWTH FACTORS**

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During the last few years 21 R-CHOP has become the standard choice of treatment for patients with B cell non-Hodgkin’s lymphoma. It has been demonstrated that this combination is effective and safe. But we observed a relatively high frequency of severe toxicity, infections, anemia and proteinuria in many patients, responsible for increased morbidity during treatment. The aim of this study was to evaluate whether the quality of life was improved after the use of profilactic granulocyte growth factors. QOL was assessed using the validated EORTC QOL-30 questionnaire, which is a 30-item instrument developed specifically for...
use in clinical cancer research. Methods: 50 patients were included between January 2008 and January 2011. 54 of them were admitted to stage I-II, and 26 stage III-IV, 42 were male and 38 female. Prophylactic use of granulocyte growth factors began after the first cycle for 40 patients and in the other group of 40, after the first neutropenia. All patients completed the QOL-C30 questionnaire at least 3 times: pretreatment, after four cycles and post-treatment. In the second group, patients completed the questionnaire at last neutropenia and before every cycle until the end of treatment. Pre-treatment global health status and role functioning were almost identical. The first group followed the treatment at exact time and the neutropenia developed did not interfere with the course of treatment. They did not develop febrile neutropenia or grade III or IV neutropenia. Therefore, global health status and role functioning were significantly decreased compared with the pre-treatment value. In contrast, in the second group, 32 patients developed neutropenia after approximately 3 cycles and global health status, physical and role functioning were significantly decreased and fatigue, dyspnoea and appetite loss significantly increased. After the introduction of granulocyte growth factors the incidence of neutropenia decreased and the appetite loss significantly increased. After the introduction of granulocyte growth factors the incidence of neutropenia decreased and the appetite loss significantly increased. After the introduction of granulocyte growth factors the incidence of neutropenia decreased and the appetite loss significantly increased.

Results: The use of prophylactic granulocyte growth factors improves QOL, except on the activity of daily living that decreased after the remission of neutropenia. After 3 months of post-treatment the patients generally scored equal. Conclusion: The use of prophylactic granulocyte growth factors improves QOL in NPHL patients treated with 21 R-CHOP and should be a standard in inclusion: The use of profilactic granulocyte growth factors improves QOL on the activity of daily living that decreased after the remission of neutropenia.

Methods: We have performed 89 semi-structured interviews with acute leukemia patients who were admitted to the Cluj Hematology Clinic during 2009 and were treated with chemotherapy. Study admission criterion was patient’s acknowledgement of religion as a psycho- logical resource. Interviews were conducted by a psycho-social counselor who is also a clinical hematologist, and were analyzed by a bioethics committee (theologian, physicians, philosopher, law specialist). Patients signed informed consent, and the study was approved by the local Ethics Committee. The interviews were analyzed qualitatively using thematic analysis. This study is a part of POSDRU/89/1.5/S/15777 Project co-financed from European Social Fund through Human Resources Development Sectoral Operational Program 2007-2013. Results: Out of the 89 patients, 37% were Christian Orthodox, 5.6% Catholics, and 6.7% Protestants. These percentages are also reflected in the general population. All patients reached an efficient coping. 30% of them experienced inefficient coping periods such as frantically praying for a miracle, questioning God’s love, hopelessness, blaming God for the disease, resignation (the disease was God’s will), or interpreting cancer as a punishment. Patterns of efficient religious coping were: keeping the faith, and following Christ’s example by combining suffering with trust- ing God. Some patients became more religious after the diagnosis, thus entering a process of spiritual awakening. They reinterpreted the disease as an opportunity for spiritual growth and study of religion. There was only one atheist patient, but his illness brought him to faith. The patients described bargaining with God as an efficient coping pattern. This is one of the phases in Elisabeth Kübler-Ross’ coping model. In bargaining the patient asks God to prolong his life or to allow him to take part in an important event in exchange for a Christian life. In our group, 38% of patients bargained for family-related matters (marrying, having children or grandchildren, seeing their children graduate), 7% bargained for profession-related matters, 2% committed to go on religious pilgrimages, and 17% did not reveal the subject of their bargaining. Some patients stated that religious coping is their patient’s personal resource, and many patients advise patients to keep hoping and to maintain a positive attitude). However, other patients displayed a passive behaviour and accepted the dis- ease as a fait accompli. Conclusion: It is important for the multidisciplinary team (oncologist, nurse, psychologist, theologian, social worker, fami- ly) that provide medical care to malignant patients to realize that religi- ous coping is not always efficient, and to recognize the most common patterns of religious coping. It is recommended that when a member of the team identifies patients with inefficient religious coping, to refer those patients for specialized psychological or spiritual counseling.
fusions and improve QoL in patients with chemotherapy induced anemia. Aims: To study the efficacy of HuEPO and improving QoL in LPD patients with anemia. Methods. There were done this prospective study to investigate the effectiveness of HuEPO in reducing RBC transfusion-dependency, increasing hemoglobin concentration and QoL in patients (n=85) with low-grade non-Hodgkin's lymphoma (n=10), chronic lymphocytic leukemia (n=21) and multiple myeloma (n=44). The median age of patients was 65.0 years (range 24-82). Recombinant human erythropoietin was injected subcutaneously on 450 IU/kg weekly. Before start of HuEPO treatment all patients have been receiving two or more cycles of antitumor chemotherapy. The patients with Hb concentration <8.0 g/dl received RBC transfusions before HuEPO treatment. The target Hb level was 12 g/dl and planned duration of HuEPO treatment within 16 weeks. Positive response was estimated as increasing Hb concentration to 20.0 g/dl or achieving target Hb level (12 g/dl) during the period of HuEPO therapy and so achieving RBC transfusion-independency. QoL was assessed using the FACT-An questionnaire. Results. Mean baseline Hb concentration was 8.7±1.5 g/dl (57-100 g/dl). Before HuEPO-therapy 24 patients had received RBC transfusion (2-17 units) during last 3-6 months because of low Hb (3.7-8.0 g/dl). The period of HuEPO-therapy was from 6 to 16 weeks (mean 9.0±3.4 weeks and median follow-up of 8.5 weeks). During the study period 7 patients (29.2%) followed RBC transfusions also after finishing of HuEPO treatment and 17 of them (70.3%) showed RBC transfusion-independency. In whole group normal Hb concentration (<12.0 g/dl) showed 39 patients (47%). While we observed positive response in 53 patients (63.9%), Hb concentration increased from baseline to 12.4±1.9 g/dl (10.5-15.4 g/dl; p<0.01). However 4 patients who didn't receive transfusions before HuEPO-therapy showed RBC transfusion-dependency because of their Hb fell down less 8.0 g/dl. The reason appearing of transfusion-dependency in these patients was progression of their diseases and so poor effectiveness anti-tumor therapy. FACT-An demonstrated that HuEPO-therapy reduced symptoms such as: fatigue, force and physical efficiency, depression, drowsiness, giddiness, headaches, pain in thorax and dyspnea. On a scale from 0 to 4 points, the symptoms reduced from 1.79 to 1.28 in positive response patients indicating an improvement in QoL. In the study patients (p<0.05; n=53). However we didn't observe any difference of the symptoms between before beginning and after finishing HuEPO-therapy in non response patients (from 1.92 to 1.98; p=0.5; n=50); their Hb concentration changed significantly. Transfusions were given to 9.0±1.99 g/dl (6.9-11.4 g/dl; p=0.1). Conclusions. The study has shown that HuEPO is effective treatment of reducing RBC transfusion-dependency, increasing Hb and improving QoL in a group of anemic patients with lymphoproliferative disorders.

1739 THE EVALUATION OF EORTC QLQ-C30 AND ITS ASSOCIATION WITH ANXIETY AND DEPRESSION IN TURKISH MULTIPLE MYELOMA PATIENTS

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Background. Multiple myeloma (MM) patients have depression, anxiety and alterations in quality of life which could be explained by disease itself and by side effects of treatment modalities. AIMS: We evaluated quality of life, depression, anxiety, and their associations with parameters like patients' personal and demographic features and stage of disease. Methods. We included 50 (15 females, 35 males) MM patients (mean age, 61.8±9.1; median disease duration, 20.7 months) diagnosed according to IMWG criteria. Patients' age, sex, disease duration, disease type and stage, treatment modalities, and other medical data were recorded from hospital files. For staging purposes, ISS was used. The performance status of the patients were evaluated by means of the ECOG performance scale. Patients were administered EORTC QLQ-C30, EORTC QLQ-MY20, General Health Questionnaire (GHQ) and Hospital Anxiety and Depression Scale (HADS). In order to compare categoric variables, Chi-square test and to compare continuous variables, unpaired-t-test were used. Pearson correlation test was utilized for correlation analysis. Multiple linear regression analysis was used to determine independent factors with the functional EORTC QLQ-C30. Results. The monoclonal protein was IgG in 22 (44%) of MM patients, IgA in 17 (34%), light chain kappa in 6 (12%) and light chain lambda in 4 (8%). Patients in early (stages I, II) and late (stage III) ISS stages were similar in functional scales, symptom scales, global QoL, subscales of EORTC QLQ-MY20, and HADS-A and HADS-D scores. When MM patients with good (ECOG 0) and poor (ECOG 2 and higher) performance status were compared, it was observed that the functional and symptom scales of EORTC QLQ-C30, global QoL, subscales of EORTC QLQ-MY20, and HADS-A and HADS-D scores were significantly different (p values <0.05). Patients with HADS-D scores >7 were accepted to have depression. The functional and symptom scales of EORTC QLQ-C30, global QoL, subscales of EORTC QLQ-MY20, and HADS-A were significantly different between patients with HADS-D scores >7 and ≤7 (p values <0.001). The physical functioning (OR, 2.53; 95%CI, 1.7-5.8; p=0.001) and role functioning (OR, 4.09; 95%CI, 2.3-45.5; p=0.03) scores of EORTC QLQ-C30 were independent factors which had positive effect on general quality of life. The treatment-related side effect score of EORTC QLQ-MY20 (OR, -3.2; 95%CI, -4.9 to -1.4; p=0.001) and the presence of depression as assessed by HADS-D (OR, -4.4; 95%CI, -2.6 to -0.4; p=0.017) were factors which had negative effect on general quality of life. Conclusions. The performance status as assessed by ECOG, presence of anxiety and depression were associated with quality of life and severity of symptoms in Turkish MM patients. The presence of depression in HADS-D was an independent prognostic parameter which had negative impact on general quality of life score. The factor which had the greatest negative influence on quality of life in MM was observed to be the treatment-related side effect score of EORTC QLQ-MY20.

1740 ERYTHROPOIETIN USE IN PATIENTS WITH AML OR UNDERGOING ALLOGENIC HSCT SIGNIFICANTLY IMPROVES QUALITY OF LIFE AND REDUCES RED BLOOD CELLS AND PLATELETS TRANSFUSIONS WITHOUT ANY SURVIVAL EFFECT

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Background: Despite frequent anemia and multiple transfusions during allo-HSCT for any hematological disease, EPO (NEORECOM® 30000UI) was administered Sc. once/week during a maximum period of 6 months; Hb level was monitored every week. Injections were stopped once Hb level reached 12g/dl without any transfusion. If after 8 injections, no improvement was observed, doses were doubled, and if after 8 injections, no improvement was observed, patient was taken off-study for EPO inefficiency. The QoL was measured at baseline, at 1, 2, 3 and 6 months by the Functional Assessment of Cancer Therapy-Anemia (FACT-An). EPO responders patients were defined as having Hb level ≥12g/dl (EPO CR) or a ≥ 2g/dl increase [EPO partial response (EPO PR)] compared with baseline value before any transfusion requirement. The matching analysis took into account: diagnosis, conditioning, HSC source, number of previous transplants and GVHD. Results: Between April 2006 and December 2009, 61 patients were included, patient characteristics are summarized in Table1. The median number of EPO injections/patient was 8 (2-28). We have noticed a trend for improvement of QoL during the 6 months follow-up according to FACT-An anemia (p=0.07). There were 71% of EPO CR after a median time of 39 days (14 - 180). After the pair-matched analysis, 44 patients were matched with at least one control patient. When comparing RBC and Pt transfusions, there were 355 units and 555 units in the matched population versus 227 and 574 and in the EPO population p=0.004 and p=0.6 respectively. The multivariate analysis on EPO CR showed the positive impact of Pt levels at baseline, the negative impact of female recipient and major ABO incompatibility. We did not find any significant difference in terms of overall (OS) and event free survival (EFS) between EPO and control group. Conclusion: We showed a positive trend of EPO administration on QoL, an achievement of a normal Hb level and a significant spare of RBC transfusions. A cost-effectiveness study is ongoing and results will be communicated.

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Cognition and Quality of Life in Adult De Novo Acute Leukemia Patients

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Background. A significant percentage of cancer patients will develop cognitive impairment, during or after completion of aggressive treatment. Cognitive deficits are usually subtle and mild, but can occur in various cognitive domains. In AML/ALL patients, impaired cognitive functions have been described, even prior to initiation of chemotherapy. In addition, classical side-effects of aggressive chemotherapy in patients with acute leukemia can have a significant impact on quality of life and on emotional aspects of well-being. Aims: To measure baseline cognitive functions and short-term cognitive evolution, and to assess depression, emotional aspects of well-being, and quality of life in de novo acute leukemia patients before and after induction treatment. Methods: Longitudinal-prospective study of adult de novo acute leukemia patients, treated with chemotherapy. This study was approved by the local Ethical Committee and informed consent for each patient was obtained. Eligible patients were enrolled and investigated with a comprehensive cognitive test battery, within five days after admission (pre-induction) and after completion of induction (pre-consolidation). Cognitive functions assessed are attention, executive functions, motor dexterity, and also verbal memory. Depression, emotional aspects of well-being, and quality of life were assessed with self-report questionnaires. Results. Twenty adult patients were enrolled between 01/2009 and 06/2010. The median age was 43 years, 50% were male, 80% had AML (20% ALL), and median duration of education was 12 years. Baseline hematological values were assessed, with WBC count (10.4 10E3/uL), RBC count (3.1 10E6/uL) and HgB (9.8 g/dL). Adult patients had normal cognitive functions (range: 1 SD below normative mean) in different cognitive domains, and mainly in attention and executive functions (COWAT, and SCWT), except for mild cognitive deficits in verbal learning (AVLT A1-5), and also especially in motor dexterity (PTT). These functions were heterogeneous at baseline, ranging from severely impaired to good within a cognitive domain. In particular, at baseline low quality of life and role functioning, and a high level of fatigue were found. Global health status related with social functioning, and role functioning related with fatigue. At follow-up, attention, executive functions, motor dexterity, and verbal learning had all improved significantly (p<0.02). Changes during chemotherapy treatment were found in depression (p<0.005), global health status (p<0.001), emotional functioning (p<0.001), and symptom scales fatigue (p=0.05) and pain (p<0.01), and all five scales of well-being (p<0.05) (CES-D, EORTC QLQ-C30, and POMS). Global health status related with four of the five functional scales, except for cognitive functioning, and with symptom scale fatigue. Global health status related with depression and vigor. Conclusions: Adult de novo acute leukemia patients exhibit at baseline normal cognitive functions, except for verbal learning and motor dexterity. Cognitive deficits in attention, executive functions, and verbal learning were found similar to previous reported research. However, our data showed less impaired motor dexterity. Moreover, cognitive functions improved at follow-up. At baseline, adult de novo acute leukemia patients have low quality of life, high levels of depression and negative status of well-being. Changes over time, were observed across global health status, dimensions of quality of life, depression, and emotional aspects of well-being.

Prospective Evaluation of Oral Mucositis by Day-by-Day Assessment in Hematological Patients Undergoing Stem Cell Transplantation

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Background. Oral mucositis (OM) is a major issue in the setting of hematopoietic stem cell transplantation (HSCT), with serious effects on quality of life. Aim. A prospective observational study was conducted in our BMT Unit to assess the pattern and severity of OM and GIM in patients (pts).

Methods. Assessment of OM in patients (pts) undergoing autologous (auto) or allogeneic (allo) HSCT was conducted daily from conditioning regimen to day +20 or discharge. OM was assessed according to WHO scale. Results. 60 HSCT (10 alloHSCT, 50 autoHSCT) were performed from January 2008 to August 2010 (high dose chemotherapy conditioning regimen), in adult patients with lymphoma (n=21), multiple myeloma (n=29), acute leukemia (n=8) or other diagnosis (n=2). All patients received high dose chemotherapy as conditioning regimen, according to the international guidelines for lymphoma or myeloma. OM prevention measures were: in allogeneic HSCT, mouth rinses alone (chlorhexidine based); in autologous HSCT, mouth rinses (chlorhexidine based) alone or associated with probiotics (Lattobacilus brevis CD2) in 45 and 7 pts, respectively. Oral criotherapy was associated in 42/50 pts undergoing intermediate-high dose melphalan-based conditioning regimen and autoHSCT. 57/60 HSCT were eligible for analysis. Data about OM are presented in table. Prevention of OM with oral criotherapy in autoHSCT (high dose melphalan conditioning in myeloma pts only) resulted on reduction of severe OM (grade 3-4). Data about OM prevention with probiotics are encouraging, although preliminary. Conclusions. Although preliminary, these data may be helpful to define the impact of OM on patients undergoing high-dose chemotherapy and evaluate preemptive and treatment approach of this complication, with the primary aim to improve patients quality of life.

Acute and Chronic Strenuous Exercises Alter Antioxidant Status in Hemoglobin E Trait Carriers

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Methods. Assessment of OM in patients (pts) undergoing autologous (auto) or allogeneic (allo) HSCT was conducted daily from conditioning regimen to day +20 or discharge. OM was assessed according to WHO scale. Results. 60 HSCT (10 alloHSCT, 50 autoHSCT) were performed from January 2008 to August 2010 (high dose chemotherapy conditioning regimen), in adult patients with lymphoma (n=21), multiple myeloma (n=29), acute leukemia (n=8) or other diagnosis (n=2). All patients received high dose chemotherapy as conditioning regimen, according to the international guidelines for lymphoma or myeloma. OM prevention measures were: in allogeneic HSCT, mouth rinses alone (chlorhexidine based); in autologous HSCT, mouth rinses (chlorhexidine based) alone or associated with probiotics (Lattobacilus brevis CD2) in 45 and 7 pts, respectively. Oral criotherapy was associated in 42/50 pts undergoing intermediate-high dose melphalan-based conditioning regimen and autoHSCT. 57/60 HSCT were eligible for analysis. Data about OM are presented in table. Prevention of OM with oral criotherapy in autoHSCT (high dose melphalan conditioning in myeloma pts only) resulted on reduction of severe OM (grade 3-4). Data about OM prevention with probiotics are encouraging, although preliminary. Conclusions. Although preliminary, these data may be helpful to define the impact of OM on patients undergoing high-dose chemotherapy and evaluate preemptive and treatment approach of this complication, with the primary aim to improve patients quality of life.
The question of whether Hb E trait carriers are exposed to exercise intolerance at biochemical (antioxidant and oxidative damage) and physiological (physical fitness) levels is addressed. First, 10 Hb E trait (21.2±6.0 yrs) and 10-paired normal Hb (21.4±0.5 yrs) participants performed a maximal oxygen uptake test (VO2max) on a treadmill. Second, 16 Hb E trait (5.1±0.9 yrs) and 10-paired normal Hb E (5.1±1.9 yrs) athletes participated in a 10-week training camp. In Hb E trait, the activity of erythrocyte glutathione peroxidase (GPx) failed to recover after 45 minutes of rest (p=0.049). In both groups of athletes, the training camp allowed to improve anaerobic capacity and maximal muscular strength but depression score was increased and VO2max was decreased. Activities of plasma and erythrocyte GPx and erythrocyte superoxide dismutase were higher at the end of the training camp compared to before (p<0.001). Plasma GPx increase was higher in Hb E (p=0.002) compared to normal Hb athletes. We conclude that Hb E trait could explain inter-individual variability in blood antioxidant markers in response to exercise. Proper training programs (suitable training load and recovery) should be of importance concerning Hb E carriers.

**1744**

**FUNCTIONAL HEALTH STATUS ASSESSMENT IN HAEMOPHILIA PATIENTS WITH THE INTERNATIONAL CLASSIFICATION OF FUNCTIONING, DISABILITY AND HEALTH (ICF, ICF-CY)**

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**Background.** People with haemophilia experience a progressive deterioration of their functional health status. Its regular clinical assessment of functional health status provides insight into their process of disablement. As such, the development of a core-set of measurement tools is warranted. **AIM:** The aim of this study was to gather data to identify clinically feasible items from existing haemophilia-specific instruments assessing the functional health status and Quality of Life (QoL) of haemophilia patients using the ICF for adults and children, (ICF-CY) frame. **Patients and methods:** Five haemophilia-specific instruments were linked to the ICF separately by 8 trained health professionals according to linking rules developed specifically for this purpose. The degree of agreement between health professionals was calculated by means of the kappa statistics. Bootstrapped confidence intervals were estimated. **Results:** Within the 5 selected instruments 365 concepts were identified, of which 223 concepts were included in the ICF and ICF CY and mapped into 70 different categories. The estimated kappa coefficients ranged between 0.85 and 0.88. 19 Nineteen categories were included for body function (27%), 4 for body structure (6%), 35 for activities and participation (50%) and 12 for environmental factors (17%). High prevalence in health areas corresponding to ICF and ICF-CY categories for activities and participation was reported for mobility, work, school, sport and social activities. Environmental factors included in the instruments were health economic aspects such as treatment, assistive products for mobility, home or life did not depend of the clinical course of the disease (such as severe anemia, bleeding, central nervous system leukemia, infections). Quality of life was significantly worse in patients who were older than 50 years old (t-test, p=0.03) and in depressed patients (p=0.0003). 60.6% of patients (n=20) met the depression criteria early after the diagnosis. After induction of remission therapy 30 patients were eligible. Most of them (n=29) achieved complete remission. Mean Quality of life score was 69.7 at this period of treatment. 46.7% of patients (n=14) reported depression. The self-reported quality of life measuring after induction of remission therapy did not depend of such complications of the treatment as bleeding, pneumonia, mucositis, hepatitis, enteropathy, nausea. These complications were successfully treated (antibiotics, anti-emetics, transfusions, etc.) Quality of life was significantly poorer in patients who had febrile neutropenia (p=0.01), age older then 50 years (p=0.006), low social support score (p=0.045), single family status (living alone) (p=0.01) and depression (p=0.001). Quality of life of patients with different education level and different income level were not significantly different (p=0.18 and p=0.7 respectively). The income level did not differ much between patients. **Conclusions.** Self-reported quality of life of newly diagnosed acute leukemia patients was significantly poorer in patients with depression or low social support level, and patients older than 50 years old. Depressive symptoms are prevalent.

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**IMPACT ON QUALITY OF LIFE OF OUTPATIENT TREATMENT IN HIGH RISK MYELODYSPLASTIC SYNDROMES: RESULTS OF A PILOT STUDY**

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**Background.** Quality of life (QoL) in myelodysplastic syndromes (MDS) depends on various factors: global health status, social setting, disease characteristics (anaemia). Moreover uncertain evolution of the disease induces emotional distress that impacts on QoL and patients experienced a high level of fatigue. Treatment of anemia with erythropoiesis stimulating agents (ESA) or hypomethylating agents, depending on the status of the disease, will improve patient functional status and quality of life. Purpose: few data are available to know if home treatment improves patient QoL. 162 patients with advanced disease were treated with chemotherapy either in outpatient or inpatient care(1). QoL was improved in outpatient group as well as social function score. In hematological issue one study including 41 patients in bone marrow transplantation2 showed benefit of home treatment in term of QoL. No data are available concerning outpatient MDS treatment. **Patients and methods:** 40 patients were included in a single center, 20 patients receiving chemotherapy at home and 20 patients at hospital, matched on gender and age. Quality of life (QoL) assessment was performed using EORTC QLQ-C30. In addition focus of control, stress and mental adjustment strategies were evaluated (using MAC-44, CLCS and stress scale). Results: mean age was 70 years. No difference was found between the 2 groups excepting stress which was higher in female than in male. 50% of patients who received treatment at hospital would like to change. At the opposite 80% of patients treated at home prefer to continue. A negative correlation was found at home between age and stress and a positive correlation between number of person at home and stress. Conclusion and perspectives: need of relevant factors which determine QoL in this population as family, caregivers, distress, and strategy of adjustment will be necessary. Larger number of patients is needed to better understand underlying conditions that could affect QoL.

**References**

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FREQUENCY AND SIGNIFICANCE OF PSYCHIATRIC DISORDERS IN HEMATOLOGICAL AND NONHEMATOLOGICAL MALIGNANCIES

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We investigated the frequency of anxious-depressive psychiatric disorders (anxiety and depression) in subjects with hematological and nonhematological malignancies; we evaluated 1051 subjects hospitalized in the period from January 1st 2003 to December 31st 2009, among them 615 (59.65%) were diagnosed with non-hematologic malignancies, 341 (32.9%) were diagnosed with malignant hematologic disease and 75 (7.35%) were diagnosed with cancer and hematologic manifestations; frequency of mental disorders in subjects with hematological malignancies was 59%, statistically significantly different (p < 0.0001) of frequency of mental disorders in subjects with depressive anxious and non hematological malignancies: 26.5%; In the subjects with hematological malignant diseases, we identified a 25% frequency of psychiatric disorders in subjects with acute myeloproliferative disorders, 40% in subjects with acute lymphoproliferative disorders, 23.5% in subjects with MDS, 38.75% in subjects with chronic myeloproliferative disorders, 46.6% in subjects with Multiple Myeloma; Note a frequency of only 21.33% of anxious depressive psychiatric disorders in subjects with para neoplastic disease (59% in hematologic malignancies, 23.53% in para neoplastic hematologic manifestations; p < 0.01). In the non-hematologic hematologic diseases lower incidence of mental disorders has been identified in autoimmune hemolytic anemia (10.3%) and highest incidence was found in Anti Phospholipid syndrome (49.5%) (p < 0.01). Impact of psychiatric disorders in non-hematological and hematological malignancies was significant in terms of quality of life, therapeutic compliance. Impact of the prognosis will be estimated by expanding the lot and monitored the dynamics analysis in terms of APL syndrome, high frequency of mental disorders is an argument for APL antibodies involvement in the pathogenesis of neuro psychiatric disorders; Phase of study conclusions indicate depressive mental disorders associated with hematological malignancies anxious to represent a significant factor influencing quality of life, therapeutic compliance and possibly prognosis of these diseases, requiring strong multidisciplinary approach.

A WAY TO REDUCE PAIN IN BONE MARROW DIAGNOSTIC PROCEDURES

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Background. Bone Marrow core biopsy and aspiration represents a standard procedure in diagnosis and staging of hematological malignancies. Attempts to reduce pain during aspiration and biopsy procedure have led to the development of new techniques such as electrically powered devices.

USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IN CHILDREN WITH CANCER: REPORT OF A BELGIAN CENTER FOR PEDIATRIC HEMATOLOGY ONCOLOGY

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Background: Complementary and alternative medicine (CAM) is a heterogeneous group of products, practices, medical and health care systems defined as not presently part of the conventional medicine. For the last decades, the use of CAM has become increasingly frequent, particularly in children with chronic illness and cancer in whom CAM is commonly reported along with conventional care. Aims: To define better the actual place of CAM in children with cancer in Belgium and to understand better the circumstances of their use and the expectations of patients and families using CAM, we have realized an observational study in children treated at the pediatric hematology oncology department of the Saint-Luc University Hospital in Brussels. Methods: A questionnaire was distributed to parents of children diagnosed with cancer between January 1st 2003 and December 31st 2009, who were not deceased or in palliative care. The participation was volunteer and the survey was sent to 301 families of patients aged from 0 to 21 years at the time of diagnosis. 153 questionnaires (50.8%) were returned and analyzed. In these, the prevalence of CAM use was 63% (55% after excluding prayer and psychotherapy). For 21.5% of users, CAM therapy was initiated prior to cancer but maintained or expanded during or after conventional treatments. 58.1% of patients used CAM for the first time during conventional treatments and 20.4% started CAM after conventional treatments. The most frequently used CAM were homeopathy (24%), massage therapy (16.2%), dietary supplements (15.6%). The most common expectations of users were an improvement of the child’s wellness (22%), a decrease of the side effects of conventional treatments (15.9%), a reinforcement of immunity (12.4%). Parents reported benefits in 90.5% of cases although negative effect was described in 10% of cases. The duration of use was 1-3 months in 13.9%, 3-6 months in 11.3%, 6 months-1 year in 18.3% and > 1 year in 46.1% of users. The frequency of use depends on the type of CAM: alternative medicines such as homeopathy were mainly used daily while manipulative and body-based methods such as massage were commonly administrated on demand. Despite a full agreement on conventional treatment, 55% of families did not disclose their CAM use to their physician. Factors as age, sex, type of tumor, place of residence or prior CAM use were not predictive for use of CAM. However, we find a significant correlation with the level of education of the mother and/or the main person in charge of the child. Conclusions: CAM is part of the treatment in a large number of children with cancer. It is commonly perceived as safe by patients and families but its undisclosed combination with conventional therapies increases the risk of drug interaction and could interfere with the analysis of clinical trials. This work underlines the necessity for physicians, CAM users and practitioners to improve their communication. Such attitude could also allow a better study of CAM efficacy.

The use of these devices may reduce pain in bone marrow aspiration. Nevertheless there is no protocolized clinical studies comparing different pain scores using this new technique. versus manual techniques. Aims: The objective of this randomized, protocolized, single blind study is to measure the seconds it takes for the needle to penetrate the skin and reach the bone marrow just before aspiration and measure the pain produced by this technique compared to manual bone marrow aspiration. Methods: 30 patients 18 men and 12 women were selected, and randomized into 2 groups. In the group 0 (n=15) powered aspiration (Vidacare battery powered device) was applied whereas in group 1 or control (n=15), aspiration was performed with a manual device (Cardinal Health Illinois Bone Marrow Needle). Every patient received subcutaneous 2% Mepivacaine in the punctation area. Time from the cortical contact to aspiration was measured in seconds. Previous pain and pain during insertion were registered according to the Patient Pain scale in which 0 is absence of pain and 10 is the most extreme pain. In order to evaluate the pain reduction a new parameter called Pain Reduction was obtained by subtracting previous pain minus pain during insertion. Statistical analysis was performed through non parametrical tests for comparison of means and results. Conclusions: The use of electrically powered device has shown in our series a significative reduction in operational biopsy time, as well as a pain reduction compared to traditional manual devices.
CHLORPROMAZINE PLUS METHOCLOPRAMIDE AND PRETOSINE IS COST-EFFECTIVE IN CONTROL OF LATE HEMESIS IN PATIENTS PRETREATED WITH PALONOSETRON THAN IN THOSE TREATED WITH TROPISERON

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Background. Late hemesis is often difficult to resolve, especially when patient received highly hemetogenic chemotherapy regimens. Data regarding the most useful drug to employ in late hemesis on the base of the type of anti HT3 drug previously used in acute hemesis (palonosetron or tropiseroton) are lacking. Aim. Aim of this study is to define which is the best cost-effective antithemetic therapy in late hemesis when palonosetron or tropiseroton are used. Results. In all patients receiving chlorpromazine. In palonosetron group, nausea and vomiting persisted, they added largactil 12.5 mg i.v. Results of Fisher exact test. A cost analysis was performed considering the median of global direct and indirect antiemetic cost. Cost were evaluated by Fisher exact test. A cost analysis was performed considering the median of global direct and indirect antiemetic cost.

RESPONSE IN OMANI PATIENTS CONTRIBUTION OF AGE, GENDER, BODY WEIGHT, CYP2C9, CYP4F2 AND VKORC1 GENOTYPE TO THE WARFARIN ANTAGOINIST RESPONSE IN OMANI PATIENTS

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Background. The study showed that although age, weight, CYP2C9, and VKORC1 polymorphisms affect the warfarin dose requirements in an individual patient, VKORC1 was the dominant predictor of warfarin dose requirement overshielding all other variables. Therefore, VKORC1 AA is the most important factor to consider while using dosing algorithms to improve safety of warfarin therapy in Omani patients.

IMPROVING STANDARDS OF CARE FOR HAEMOGLOBINOPATHIES IN A LOW PREVALENCE REGION

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Births of patients with major Haemoglobin disorders tend to be concentrated in a few urban areas throughout the UK. This has led to the development of some highly specialised centres for the care of patients in these areas. However in areas of lower prevalence, services may be poorly developed, coordinated and funded, leading to inequalities in care provision. We report an initiative by the East Midlands Commissioning Team, to support a multidisciplinary hub and spoke network of services for patients throughout the East Midlands, with outreach services from two linked centres to surrounding areas. The £250k package supported the appointment of a data manager, two WTE hospital based nurse specialists and support for roll out of the regional transcranial Doppler (TCD) and psychology support services. Within a year a regional database has been established, >50% patients have been recruited to the newly established National Haemoglobinopathy Register, the EMidlands TCD service has achieved 100% coverage of the main centres, with outreach service for small centres available from 2011, development and access to agreed regional guidelines for acute management of sickle crises, positive report of nationally run peer review of children’s services, impact of nurses on outpatient non-attendance, as well as an annual report to commissioners. Novel approaches to the use of scarce resources are being developed (eg screening assessment tools for referral to psychology services) and dedicated regional audit and education material. The process has contributed to the concept of developing a national commissioning tool for haemoglobinopathy services, which will outline standards of care and services that must be provided in all areas. These will be available for use by any commissioning structure that emerges from NHS reforms in the UK.
from request forms, patients notes and laboratory electronic data. Results. There were of 321 requests 139 (43%) were form A&E, 52 (17%) from ICU, 51 (16%) were from medical, 40 (12%) were form surgical, 24 (7%) were from paediatrics and 15 (5%) were from obstetric and gynaecology. 163 (51%) had irrelevant clinical details and 96 (30%) had relevant information. There were no clinical details available on 62 (19%) requests, most commonly from A&E (46%). Only 49 (15%) screens yielded abnormal results. Conclusions. These results indicate a large number of irrelevent requests. They also show a considerable amount of forms either with no clinical details or irrelevant information. Such unnecessary or poorly informed requests waste a significant laboratory and clinical time and could potentially raise the need for further detailed investigations. Eventually this can result in more costs, delay in patients’ discharge and further unnecessary stress to patients and workload to staff. Moreover lack of proper clinical details and irrelevant requests would affect the outcome of sample authorization reports by haematologists.

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AN AUDIT OF COAGULATION SPECIMENS REQUIRING DIRECT HUMAN INVOLVEMENT AT AN ACADEMIC LABORATORY
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Background. Currently there is an increasing trend towards automation in the laboratory testing of coagulation. Some coagulation specimens cannot be summarily tested in currently utilized fully automated systems because of suboptimal pre-analytical influences, such as under-filled tubes. More accurate results would yield spurious results. Other specimens require additional sample preparation, such as double centrifugation, before testing. Aims. To determine what proportion of specimens received by the coagulation section of the Universitas Academic Laboratory required direct human involvement for screening and/or additional sample preparation prior to testing and to analyze the contributing factors. Methods: A random sample of 569 specimens received for coagulation testing from 1 December 2008 to 30 September 2009 were audited. Specimens inspected by technologists and found to be under-filled, clotted, collected in incorrect specimens tubes, lipaemic, icteric, haemolysed, from patients with haematocrits >0.55; tubes: 127 (1.5%); lipemic: 8 (0.1%); icteric: 43 (0.5%); haemolysed: 75 (0.9%); lupus anticoagulant: 347 (4.0%); activated protein-C resistance (APCR) testing (both requiring double centrifugation), were labeled, and specimens for lupus anticoagulant or activated protein-C resistance (APCR) testing (both requiring double centrifugation), were only 49 (15%) screens yielded abnormal results. Conclusions. These results indicate a large number of irrelevant requests. They also show a considerable amount of forms either with no clinical details or irrelevant information. Such unnecessary or poorly informed requests waste a significant laboratory and clinical time and could potentially raise the need for further detailed investigations. Eventually this can result in more costs, delay in patients’ discharge and further unnecessary stress to patients and workload to staff. Moreover lack of proper clinical details and irrelevant requests would affect the outcome of sample authorization reports by haematologists.

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MICROSCOPIC EXAMINATION OF BONE MARROW ASPIRATION SMEARS: DIAGNOSTIC AGREEMENT OF HEMATOLOGISTS AND HEMATOPATHOLOGISTS ON COMMON HEMATOLOGICAL DIAGNOSES
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Background. Morphological examination of bone marrow aspiration slides is one of the routine and the most valuable diagnostic tools to evaluate hematological disorders for the hematologists. Hematopathologists evaluate those in combination with more complicated techniques such as immunohistochemistry. However, there are no data about the agreement levels on microscopic evaluation of bone marrow aspiration slides between hematologists and hematopathologists. Aims. To determine the agreement levels between hematologists and hematopathologists on common hematological diagnoses. Methods. A random sample of 569 patients who underwent bone marrow aspiration procedure with or without bone marrow biopsy for clinical purposes between January 2008 and December 2009 were chosen. Archived Wright-Giemsa-stained bone marrow aspiration smears and corresponding pathology reports belonging to those patients were reviewed. Patients who had an adequate bone marrow aspiration smear and corresponding hematopathology report were included into the study (n=525). Two-hematology fellow and a consultant hematopathologist with a 12, 24, and 120-month experience on microscopic evaluation, respectively, were assigned to evaluate smears. Hematologists evaluated 15 bone marrow smears per week during their training period. In order to determine inter-observer agreement, hematologists reviewed 10 smears. The diagnoses of smears were obtained from hematopathology reports. For each slide, brief clinical and laboratory information was provided. Hematologists gave a diagnostic code for every slide according to predefined diagnoses based on hematopathology reports. A good inter-observer agreement was achieved between the hematologists. Then a pilot test was performed in order to test the feasibility of coding system and to accommodate hematologists to coding system. Hematologists reviewed 30 smears and coded every slide for one diagnosis. An acceptable agreement was observed between hematologists and hematopathologist. At last, hematologists evaluated all 325 slides. Agreement between hematologists and hematopathology reports was assessed (Figure 1). Agreement analyses were performed by Cohen’s kappa, Fleiss kappa, and AC1 statistics. Kappa and AC1 values were interpreted as follows: less than 0.20, poor; 0.20 to 0.40, fair; 0.40 to 0.60, moderate; 0.60 to 0.80, good; and 0.80 to 1.00, very good agreement. Results. Overall agreement between hematologists and hematopathologist was good (κ: 0.76, AC1: 0.78). Hematologists were especially successful for the diagnoses of acute leukemia, multiple myeloma, and chronic myeloproliferative neoplasias with excellent agreement levels (Kappa: 0.91, AC1: 0.90; kappa: 0.92, AC1: 0.91; and kappa: 0.91, AC1: 0.91, respectively). Diagnosis of iron deficiency anemia by bone marrow aspiration smear was a challenge (κ: 0.31, AC1: 0.22). Agreement for the other hematological diagnoses was acceptable (Figure 2).

Conclusions. Microscopic evaluation of the bone marrow smears performed by hematologists gives mostly reliable and valuable results. This is especially important for the diagnosis of acute and life-threatening conditions such as acute leukemia. Assessment of bone marrow smears as well as basic history, physical examination, laboratory data, and flow cytometry results would help in the stage of diagnosis made by hematopathologists. Routine use of iron staining in patients with anemia may increase the diagnostic power of bone marrow smear when used by hematologists.
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