

## Cover Figure

Strong cytoplasmic-restricted ALK positivity in Phoenix cells transfected with EML4-ALK. This illustration is taken from the article by Sozzi et al. on page 1307.

## Editorials and Perspectives

- 1185 **Genetic variation in hepcidin expression and its implications for phenotypic differences in iron metabolism**  
Henry K. Bayele, and Surjit Kaila S. Srail

At the core of iron homeostasis is hepcidin, a small acute phase antimicrobial peptide that now also appears to synchronously orchestrate the response of iron transporter and regulatory genes. In this perspective article, Drs Bayele and Srail discuss cis and trans acting factors that may influence hepcidin variation in humans and their potential role in iron metabolism control. See related papers on page 1293 and 1297.

- 1188 **Hypereosinophilic syndrome variants: diagnostic and therapeutic considerations**  
Florence Roufousse

Hypereosinophilic syndromes are a group of disorders characterized by persistent and marked hypereosinophilia not due to an underlying disease known to cause eosinophil expansion, and which is directly implicated in damage or dysfunction of at least one target organ or tissue. In this perspective article, Dr. Roufousse provides an updated classification of these disorders and discusses the recent advances in this field, including fascinating pathogenic mechanisms and novel targeted therapeutic approaches. See related paper on page 1236.

- 1194 **First-line therapy of CD20<sup>+</sup> diffuse large B-cell lymphoma: facts and open questions**  
Ercole Brusamolino

CHOP chemotherapy, administered every 21 days, has been for years the standard therapy for advanced diffuse large B-cell lymphoma, with a long-term overall survival rate of about 40%. In this perspective article, Dr. Brusamolino discusses the recent advances in the treatment of this condition. See related paper on page 1250.

- 1198 **Immune dysfunction in chronic lymphocytic leukemia T cells and lenalidomide as an immunomodulatory drug**  
Alan G. Ramsay and John G. Gribben

A new agent that is being used in chronic lymphocytic leukemia (CLL) and is receiving considerable interest is the immunomodulatory drug lenalidomide. The precise anti-CLL mechanism of action of lenalidomide is not yet completely defined. In this perspective article, Drs. Ramsay

and Gribben examine the multiple biological effects of lenalidomide on various cell targets that likely contribute to its anti-CLL activity. See related paper on page 1266.

## Original Articles

- 1203 **Red Cell Disorders**  
**First mutation in the red blood cell-specific promoter of hexokinase combined with a novel missense mutation causes hexokinase deficiency and mild chronic hemolysis**  
Karen M.K. de Vooght, Wouter W. van Solinge, Annet C. van Wesel, Sabina Kersting, and Richard van Wijk

The red cell enzyme Hexokinase (HK), which catalyzes the phosphorylation of glucose to glucose-6-phosphate, is transcribed by an erythroid-specific promoter. Studying the molecular defect of a patient with hemolytic anemia due to HK deficiency, the authors identified the first mutation affecting the erythroid transcription of the HK gene and show its consequences in vivo.

- 1211 **Thalassemia Syndromes**  
**Imbalanced globin chain synthesis determines erythroid cell pathology in thalassemic mice**  
Kanitta Srinoun, Saovaras Svasti, Worrakavee Chumworathayee, Jim Vadolas, Phantip Vattanaviboon, Suthat Fucharoen, and Pranee Winichagoon

Globin chain synthesis imbalance is the hallmark of thalassemia syndromes. In this study the authors have cross-bred human  $\beta^{V61I-654}$  knock-in thalassemic mice with transgenic animals carrying four copies of the human  $\beta^0$ -globin gene and analyzed their progeny. The presence of multiple copies of the  $\beta^0$ -globin transgene significantly improved the globin chain synthesis ratio and the hematological phenotype of thalassemic mice.

- 1220 **Bone Marrow Failure**  
**Incidence and risk factors of aplastic anemia in Latin American countries: the LATIN case-control study**  
Eliane Maluf, Nelson Hamerschlag, Alexandre Biasi Cavalcanti, Alvaro Avezum Júnior, José Eluf-Neto, Roberto Passetto Falcão, Irene G. Lorand-Metze, Daniel Goldenberg, César Leite Santana, Daniela de Oliveira Werneck Rodrigues, Leny Nascimento da Motta Passos, Luis Gastão Mange Rosenfeld, Marimília Pitta, Sandra Loggeto, Andreza A. Feitosa Ribeiro, Elvira Deolinda Velloso, Andrea Tiemi Kondo, Erika Oliveira de Miranda Coelho, Maria Carolina Tostes Pintão, Hélio Moraes de Souza, José Rafael Borbolla, and Ricardo Pasquini

Associations between aplastic anemia and numerous drugs, pesticides and chemicals have been reported. This study conducted in Latin American countries shows a low



incidence of aplastic anemia in this region of the world. Frequent exposure to benzene-based products increases this risk, while any association with specific drugs is uncertain.

- 1227 **Chronic Myeloid Leukemia**  
**A co-operative evaluation of different methods of detecting BCR-ABL kinase domain mutations in patients with chronic myeloid leukemia on second-line dasatinib or nilotinib therapy after failure of imatinib**  
 Thomas Ernst, Franz X. Gruber, Oliver Pelz-Ackermann, Jacqueline Maier, Markus Pfirrmann, Martin C. Müller, Ingvild Mikkola, Kimmo Porkka, Dietger Niederwieser, Andreas Hochhaus, and Thoralf Lange

Various techniques have been employed to detect BCR-ABL kinase domain mutations in patients with chronic myeloid leukemia who are resistant to imatinib. The findings of this study suggest that denaturing high performance liquid chromatography combined with direct sequencing is a reliable screening technique for the detection of BCR-ABL kinase domain mutations.

- 1236 **Myeloproliferative Neoplasms**  
**T-cell abnormalities are present at high frequencies in patients with hypereosinophilic syndrome**  
 Grzegorz Helbig, Agata Wiczorkiewicz, Joanna Dziachkowska-Suszek, Mirosław Majewski, and Sławomira Kyrzcz-Krzemien

A T-cell clone, identified by clonal rearrangement of the T-cell receptor and by the presence of aberrant T-cell immunophenotype in peripheral blood, defines lymphocytic variant of hypereosinophilic syndrome. This study shows that T-cell abnormalities are present at high frequencies in patients with hypereosinophilic syndrome. See related perspective article on page 1188.

- 1242 **Acute Promyelocytic Leukemia**  
**Central nervous system involvement at first relapse in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monochemotherapy without intrathecal prophylaxis**  
 Pau Montesinos, Joaquín Díaz-Mediavilla, Guillermo Debén, Virginia Prates, Mar Tormo, Vicente Rubio, Inmaculada Prez, Isolda Fernández, Maricruz Viguria, Chelo Rayón, José González, Javier de la Serna, Jordi Esteve, Juan M. Bergua, Concha Rivas, Marcos González, Jose D. González, Silvia Negri, Salut Brunet, Bob Lowenberg, and Miguel A. Sanz

With the advent of more effective therapy for the bone marrow in acute promyelocytic leukemia (APL) central nervous system (CNS) prophylaxis has been suggested to be useful. Using data from 739 patients treated on two Spanish national trials, the authors examine the incidence of CNS relapse in APL and whether risk factors for such relapse can be identified.

- 1250 **Malignant Lymphomas**  
**Dose-dense and high-dose chemotherapy plus rituximab with autologous stem cell transplantation for primary treatment of diffuse large B-cell lymphoma with a poor prognosis: a phase II multicenter study**  
 Umberto Vitolo, Annalisa Chiappella, Emanuele Angelucci, Giuseppe Rossi, Anna Marina Liberati, Maria Giuseppina Cabras, Barbara Botto, Giovannino Ciccone, Gianluca Gaidano, Lorenzo Falchi, Roberto Freilone, Domenico Novero, Lorella Orsucci, Vincenzo Pavone, Enrico Pogliani, Delia Rota-Scalabrini, Flavia Salvi, Anna Tonso, Alessandra Tucci, and Alessandro Levis on behalf of Gruppo Italiano Multiregionale Linfomi e Leucemie (GIMURELL)

Patients with diffuse large B-cell lymphoma with an intermediate/high or high-risk according to the age-adjusted International Prognostic Index have a dismal prognosis. This clinical trial suggests that the addition of rituximab to high-dose chemotherapy is effective and safe in diffuse large B-cell lymphoma with a poor prognosis. See related perspective article on page 1194.

- 1259 **Chronic Lymphocytic Leukemia**  
**Improved survival in chronic lymphocytic leukemia the past decade: a population-based study including 11,179 patients diagnosed 1973-2003 in Sweden**  
 Sigurdur Y. Kristinsson, Paul W. Dickman, Wyndham H. Wilson, Neil Caporaso, Magnus Björkholm, and Ola Landgren

Clinical management of chronic lymphocytic leukemia (CLL) patients has changed considerably over the last years, reflected in an increased use of prognostic markers, new therapeutic agents and procedures, and supportive care measures. In this large population-based cohort study of over 11,000 CLL patients diagnosed in Sweden between 1973 and 2003, the authors found significantly improved 5-year and, most importantly 10-year CLL survival trends in all age groups. The observed improvements are likely due to improved therapeutic developments and supportive care.

- 1266 **Chronic Lymphocytic Leukemia**  
**Lenalidomide-induced upregulation of CD80 on tumor cells correlates with T-cell activation, the rapid onset of a cytokine release syndrome and leukemic cell clearance in chronic lymphocytic leukemia**  
 Georg Aue, Ndegwa Njuguna, Xin Tian, Susan Soto, Thomas Hughes, Berengere Vire, Keyvan Keyvanfar, Federica Gibellini, Janet Valdez, Carol Boss, Leigh Samsel, J. Philip McCoy Jr, Wyndham H. Wilson, Stefania Pittaluga, and Adrian Wiestner

In chronic lymphocytic leukemia (CLL) lenalidomide causes striking immune activation, possibly leading to clearance of tumor cells. This study shows that upregulation of CD80 on tumor cells and T-cell activation, which appears to be dispensable for the drug's anti-tumor



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effects, correlate with unique toxicities of lenalidomide in CLL. This provides a rationale for combinations of lenalidomide with immunosuppressive agents. See related perspective article on page 1198.

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**Multiple Myeloma**  
**A polymorphism in NFKB1 is associated with improved effect of interferon- $\alpha$  maintenance treatment of patients with multiple myeloma after high-dose treatment with stem cell support**

Annette J. Vangsted, Tobias W. Klausen, Peter Gimsing, Niels F. Andersen, Niels Abildgaard, Henrik Gregersen, and Ulla Vogel

Interferon-alpha as maintenance therapy after high-dose therapy for multiple myeloma has been intensively debated during the last 30 years because several clinical studies have been published with conflicting results. In the present study, the authors addressed the question, of whether inborn variation in genes involved in inflammation influence treatment outcome in multiple myeloma patients receiving interferon-alpha as maintenance therapy. The findings suggest that a polymorphism in NFKB1 may be associated with improved effect of interferon- $\alpha$  in multiple myeloma patients, with homozygous carriers of the wild type allele having longer survival.

1282

**Stem Cell Transplantation**  
**Is mobilized peripheral blood comparable with bone marrow as a source of hematopoietic stem cells for allogeneic transplantation from HLA-identical sibling donors? A case-control study**

David Gallardo, Rafael de la Cámara, Jose B. Nieto, Ildefonso Espigado, Arturo Iriando, Antonio Jiménez-Velasco, Carlos Vallejo, Carmen Martín, Dolores Caballero, Salut Brunet, David Serrano, Carlos Solano, Josep M. Ribera, Javier de la Rubia, and Enric Carreras

The question of the relative efficacy of stem cell sources (bone marrow vs peripheral blood) for sibling allografts still remains, particularly in relation to quality of life. A study of a relatively homogeneous population has confirmed similar outcomes in terms of overall survival, transplant-related mortality or relapse incidence. However acute and chronic graft-vs-host disease showed increases in the peripheral blood group. Possibly as a consequence, although global quality of life did not differ, there was also a significant impairment of role and social functioning in this group.

**Brief Reports**

1289

**Thalassemia Syndromes**  
**Two new  $\beta$ -thalassemia deletions compromising prenatal diagnosis in an Italian and a Turkish couple seeking prevention**

Marjon Phylipsen, Antonio Amato,

Maria Pia Cappabianca, Jan Traeger-Synodinos, Emmanuel Kanavakis, Nazli Basak, Renzo Galanello, Teresa Tuveri, Giovanni Ivaldi, Cornelis L. Harteveld, and Piero C. Giordano

Two novel deletions in the beta gene cluster were identified by Multiplex Ligation-dependent Probe Amplification in two at-risk couples seeking prevention. This study exemplifies a successful diagnostic approach in case one member of the couple is an atypical thalassemia carrier.

1293

**Thalassemia Syndromes**  
**Association of hepcidin promoter c.-582  $\alpha>\gamma$  variant and iron overload in thalassemia major**

Marco Andreani, Francesca Clementina Radio, Manuela Testi, Carmelilia De Bernardo, Maria Troiano, Silvia Majore, Pierfrancesco Bertucci, Paola Polchi, Renata Rosati, and Paola Grammatico

The liver peptide hepcidin is the key regulator of iron homeostasis. This report shows that liver iron content and serum ferritin levels are greater in thalassemia patients with the -582G polymorphic change in the hepcidin promoter as compared with patients with the wild-type (A) sequence. This observation suggests that this change might influence the iron status of irregularly chelated thalassemic patients. See related perspective article on page 1185.

1297

**Disorders of Iron Metabolism**  
**Time-course analysis of serum hepcidin, iron and cytokines in a C282Y homozygous patient with Schnitzler's syndrome treated with interleukin-1 receptor antagonist**

Marcel van Deuren, Joyce J.C. Kroot, and Dorine W. Swinkels

This report show that a Schnitzler' syndrome patient, who was homozygous for HFE C282Y hemochromatosis, responded to endogenous inflammatory cytokine production by increasing hepcidin levels, and that such increased levels were lowered by treatment with a interleukin-1 receptor antagonist. This study clearly shows in vivo that the C282Y mutation does not abrogate cytokine-induced hepcidin synthesis, and suggests that chronic inflammation may modulate iron stores in HFE hemochromatosis. See related perspective article on page 1185.

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**Acute Myeloid Leukemia**  
**Single nucleotide polymorphism genomic arrays analysis of t(8;21) acute myeloid leukemia cells**

Tadayuki Akagi, Lee-Yung Shih, Seishi Ogawa, Joachim Gerss, Stephen R. Moore, Rhona Schreck, Norihiko Kawamata, Der-Cherng Liang, Masashi Sanada, Yasuhito Nannya, Stefan Deneberg, Vasilios Zachariadis, Ann Nordgren, Jee Hoon Song, Martin Dugas, Sören Lehmann, and H. Phillip Koefler

Translocation of chromosomes 8 and 21, t(8;21), resulting in the AML1-ETO fusion gene, is associated with acute myeloid leukemia (AML). The findings of this study indicate that genomic alterations and KIT-D816 mutation confer a poor prognosis in t(8;21) AML patients.



- 1307 **Malignant Lymphomas**  
The *EML4*-ALK transcript but not the fusion protein can be expressed in reactive and neoplastic lymphoid tissues  
*Gabriella Sozzi, Maria Paola Martelli, Davide Conte, Piergiorgio Modena, Valentina Pettirossi, Stefano A. Pileri, and Brunangelo Falini*

*In the search for driver mutations that contribute to the development of cancer, translocations of ALK-EML4 were implicated as specific to non-small cell lung cancer. The author of this study clearly question the specificity of the rearrangement, showing that it is present in reactive lymph nodes and a number of different lymphomas.*

- 1312 **Stem Cell Transplantation**  
Improving outcome of patients older than 30 years receiving HLA-identical sibling hematopoietic stem cell transplantation for severe acquired aplastic anemia using fludarabine-based conditioning: a comparison with conventional conditioning regimen  
*Sébastien Maury, Andrea Bacigalupo, Paolo Anderlini, Mahmoud Aljurf, Judith Marsh, Gérard Socié, Rosi Oneto, and Jakob R. Passweg on behalf of the Severe Aplastic Anemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT-SAAWP)*

*Upfront or second line allogeneic hematopoietic stem cell transplantation (HSCT) offers a good disease-free survival option for patients suffering from severe idiopathic aplastic anemia. This report shows improved outcome of reduced-intensity versus conventional conditioning in HLA-identical sibling HSCT for older aplastic anemia patients.*

- 1316 **Cell Therapy and Immunotherapy**  
Retroviral transfer of human CD20 as suicide gene for adoptive T-cell therapy  
*Marieke Griffioen, Esther H.M. van Egmond, Michel G.D. Kester, Roel Willemze, J.H. Frederik Falkenburg, and Mirjam H.M. Heemskerk*

*The aim of adoptive T-cell therapy of cancer is to selectively confer immunity against tumor cells. Autoimmune side effects, however, remain a risk, emphasizing the relevance of a suicide mechanism allowing in vivo elimination of infused T cells. The findings of this study support the broad value of human CD20 as suicide gene in T-lymphocytes and safety switch in adoptive T-cell therapy.*

## Letters to the Editor

- 1321 **Erythrocytosis**  
Identification of high oxygen affinity hemoglobin variants in the investigation of patients with erythrocytosis  
*Melanie J. Percy, Nauman N. Butt, Gerard M. Crotty, Mark W. Drummond, Claire Harrison, Gail L. Jones, Matthew Turner, Jonathan Wallis, and Mary Frances McMullin*
- 1322 **Acute Lymphoblastic Leukemia**  
Pyruvate kinase M2 and prednisolone resistance in acute lymphoblastic leukemia  
*Esther Hulleman, Mathilde J.C. Broekhuis, Rob Pieters, and Monique L. Den Boer*
- 1324 **Acute Leukemia**  
Detection of twelve nucleotides insertion in the BCR-ABL kinase domain in an imatinib-resistant but dasatinib-sensitive patient with biphenotypic acute leukemia  
*Sandrine Hayette, Kaddour Chabane, Andrei Tchirkov, Marc G. Berger, Franck E. Nicolini, and Olivier Tournilhac*
- 1326 **Platelet Disorders**  
Elevated profile of Th17, Th1 and Tc1 cells in patients with immune thrombocytopenic purpura  
*Jingbo Zhang, Daoxin Ma, Xiaojuan Zhu, Xun Qu, Chunyan Ji, and Ming Hou*
- 1329 **Stem Cell Transplantation**  
Second bone marrow transplantation for patients with thalassemia: risks and benefits  
*Polina Stepensky, Reuven Or, Michael Y. Shapira, Shoshana Revel-Vilk, Jerry Stein, Igor B. Resnick*

## Obituary

- 1331 **Jean Dausset a scientific pioneer: intuition and creativity for the patients (1916-2009)**  
*Laurent Degos*

## Continuing Medical Education

The effect of source of hematopoietic stem cells for transplantation from HLA-identical sibling donors

Introducing rituximab into primary treatment schemes for the poor prognosis diffuse large B-cell lymphoma

T-cell abnormalities in patients with hypereosinophilic syndrome

Central nervous system involvement in patients with acute promyelocytic leukemia