Chromosome 1 abnormalities in newly diagnosed elderly multiple myeloma patients treated with novel therapies

by Simona Caltagirone, Marina Ruggeri, Simona Aschero, Milena Gilestro, Daniela Oddolo, Francesca Gay, Sara Bringhen, Caterina Musolino, Luca Baldini, Pellegrino Musto, Maria T. Petrucci, Gianluca Gaidano, Roberto Passera, Benedetto Bruno, Antonio Palumbo, Mario Boccadoro, and Paola Omedè

Haematologica 2014 [Epub ahead of print]

doi:10.3324/haematol.2014.103853

Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Chromosome 1 abnormalities in newly diagnosed elderly multiple myeloma patients treated with novel therapies

RUNNING HEAD: Chr1 abnormalities in MM treated with new drugs

Simona Caltagirone1,8, Marina Ruggeri1, Simona Aschero1, Milena Gilestro1, Daniela Oddolo1, Francesca Gay1, Sara Bringhen1, Caterina Musolino3, Luca Baldini3, Pellegrino Musto4, Maria T. Petrucci5, Gianluca Gaidano6, Roberto Passera7, Benedetto Bruno1, Antonio Palumbo1, Mario Boccadoro1 and Paola Omedè1

1Division of Hematology, University of Torino, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Torino, Italy;
2Division of Hematology, Department of General Surgery and Oncology, A.O.U. Policlinico G. Martino, Messina, Italy;
3Division of Hematology, Fondazione IRCCS Ca Granda, OM Policlinico, University of Milano, Milano, Italy;
4Scientific Direction, IRCCS – CROB, Referral Cancer Center of Basilicata, Rionero in Vulture (Pz), Italy;
5Division of Hematology, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Torino, Italy;
6Division of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy;
7Division of Nuclear Medicine, University of Torino, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Torino, Italy;
8Clinical Pathology, School of Medicine, University of Torino, Torino, Italy.

AUTHORSHIP CONTRIBUTION
All authors provided substantial contributions in study conception and in the acquisition, analysis or interpretation of data. They also revised the article at any stage and finally approved the version to be published. S.C., M.R., S.A., M.G. and D.O. mainly performed the laboratory analyses (multiparameter flow cytometry, plasma cells purification, interphase fluorescence in situ hybridization). S.C. and M.R. mainly designed the FISH and immunophenotypic study, collected/merged/analyzed all the data and wrote the manuscript. P.O. supervised the project. R.P. performed statistical analyses. F.G., S.B., C.M., L.B., P.M., M.T.P., G.G., B.B., A.P., M.B. were involved in the trial design, patients enrolment and clinical data collection and analysis.

CORRESPONDENCE
Paola Omedè, Division of Hematology, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Via Genova 3, 10156 Torino, Italy, E-mail: paolaomede@yahoo.com

TRIAL REGISTRATION: www.clinicaltrials.gov = #NCT01063179.
ACKNOWLEDGMENTS
The authors would like to thank Fondazione Neoplasie Sangue – Onlus (FO.NE.SA.) that support this research in the form of salary for the laboratory personnel.

Abstract

Multiple Myeloma is a plasma cell disorder, characterized by malignant plasma cell infiltration in the bone marrow, serum and/or urine monoclonal protein and organ damage. The aim of this study was to investigate the impact of chromosome 1 abnormalities in a group of elderly (>65 years) newly diagnosed Multiple Myeloma patients, enrolled in the GIMEMA-MM-03-05 trial and treated with bortezomib, melphalan and prednisone vs bortezomib, melphalan, prednisone and thalidomide followed by bortezomib and thalidomide maintenance. We also evaluated the link between chromosome 1 abnormalities and other clinical, genetic and immunophenotypic features by a multivariate logistic regression model. Interphase fluorescence in situ hybridization on immunomagnetically purified plasma cells and bone marrow multiparameter flow cytometry were employed. By a multivariate Cox model, chromosome 1 abnormalities, age >75 and CD19+/CD117- bone marrow plasma cells immunophenotype emerged as independent risk factors for Overall Survival in elderly, newly diagnosed multiple myeloma patients. Moreover, a detrimental effect of thalidomide, even when administered in association with bortezomib, was observed in abnormal chromosome 1 as well as (17p)deleted patients, while the benefit of thalidomide addiction to the bortezomib-melphalan-prednisone regimen was noted in patients carrying an aggressive CD19+/CD117- bone marrow plasma cells immunophenotype. This trial was registered at www.clinicaltrials.gov as #NCT01063179.
Introduction

Multiple Myeloma (MM) is a plasma cell (PC) disorder characterized by the expansion of clonal PC in the bone marrow (BM) (>10%), the detection of monoclonal immunoglobulins (Ig) in serum and/or urine and the presence of organ damage. Two thirds of the patients are older than 65 years. In Europe, approved therapy for elderly patients or patients not eligible for transplantation is currently based on melphalan (M) and prednisone (P) with thalidomide (T) or bortezomib (V). Recent studies show that lenalidomide, in association with MP or dexamethasone, is a valid alternative. Despite the introduction of novel agents in clinical practice, the outcome greatly differs among patients and new prognostic factors are needed to allow patient stratification by risk and personalized treatment.

Multiparameter Flow Cytometry (MFC) is widely used to characterize BMPC and its impact to define patients’ prognosis has largely been investigated by several authors. MFC is currently the main tool for minimal residual disease evaluation during follow-up, while, at diagnosis, cytogenetic abnormalities represent powerful prognostic factors together with the International Staging System (ISS).

Interphase Fluorescence In Situ Hybridization (iFISH) allows the identification of the most important genetic aberrations, such as deletion of Rb1 [del(13)], p53 [del(17p)], 1p [del(1p)], gain(1q) and IgH translocations. In a previous study two hierarchic groups of MM patients were identified with different prognostic impact: the "high-risk group", based on the presence of at least one among del(17p), t(4;14)(p16;q32) or t(14;16)(q32;q23) and the "standard-risk group", characterized by the absence of any of the above mentioned abnormalities.

Several other chromosomal aberrations have been investigated and gain(1q) has been identified as one of the most recurrent genetic events (>50%). Gain(1q) has been recently included in a new cytogenetic classification based on iFISH analysis: “adverse iFISH”, defined by the presence of one or more of the following aberrations: gain(1q), t(4;14), t(14;16), t(14;20)(p12-p21;q32), del(17p); “favourable iFISH”, characterized by the absence of these cytogenetic abnormalities and/or by the
presence of hyperdiploidy, t(6;14)(p12-p21;q32) or t(11;14)(q13;q32). Del(1p) represents a quite rare event (<10%) and is considered an adverse prognostic factor in young patients. The relevance of chr1 abnormalities is reported in several studies: Shaughnessy et al. defined a 70-gene high-risk signature, in which 30% of genes mapped to chr1, suggesting the significant poor prognostic impact of gain(1q) and del(1p). Moreover, CKS1B overexpression at 1q21 and its involvement in aggressive disease has been described. Leone et al. focused on CDKN2C deletion, at 1p32.3, which greatly affects cell-cycle regulation and MM pathogenesis. Despite the considerable number of molecular and clinical studies on gain(1q), del(1p) or both, the real role of chr1 abnormalities in MM remains a matter of debate. As far as gain(1q) is concerned, the poor prognostic impact of this aberration has been demonstrated in several patients series: (1) in newly diagnosed patients, enrolled in the CMG2002 trial, treated with high-dose chemotherapy and autologous stem cell transplantation; (2) in patients with recurrent disease, treated with lenalidomide and dexamethasone; (3) in relapsed or refractory patients treated with V. The efficacy of T-based regimens has been recently investigated on both newly diagnosed and relapsed/refractory MM carrying gain(1q21), demonstrating that T is not capable of overcoming gain(1q) adverse influence on survival.

This retrospective study examines the clinical impact of chr1 aberrations, other common cytogenetic abnormalities and PC immunophenotype in a large series of elderly, newly diagnosed MM patients, enrolled in a phase III randomized trial comparing VMP versus VMPT followed by VT maintenance (VMPT-VT).
Methods

Patients
Between 2006 and 2009, 511 elderly (>65 years) untreated MM patients from 61 Italian Hematology Centers were enrolled in a phase III randomized clinical trial comparing VMP vs VMPT-VT\textsuperscript{31,32}. Patients gave written, informed consent before entering the study, performed according to the Declaration of Helsinki (Ethics Committee approval number 163/0057512). 399 BM samples were centralized to our laboratory and analyzed by MFC. 376/399 samples were purified for routine iFISH analysis. The amount of BMPC allowed for the evaluation of chr1 abnormalities in 278/376 patients.

Immunophenotype
Four-color MFC was performed using CD38 APC, CD138 FITC, CD20 APC, CD45 PerCP, CD19 PerCP-Cy5.5, cytoplasmic k FITC and λ PE (BD Biosciences); CD117 PE, CD56 PE, (Caltag Laboratories), monoclonal antibodies. A FACSCalibur flow cytometer was used for data acquisition, and CELL Quest Pro Software for analysis. An antigen was positive when >30% of BMPC expressed it on cell surface.

BMPC Sorting
BMPC were enriched using anti-CD138-coated magnetic MicroBeads and AutoMACS Pro Separator (Miltenyi Biotech) following manufacturer's instructions, then fixed in Carnoy’s solution. Purity was assessed by MFC (PC always exceeded 90%).

iFISH
iFISH was performed according to manufacturer’s instructions. Probes for 1p32, Rb1 (on 13q14), and p53 (on 17p13.1) deletions; 1q21 gain and t(11;14)(q13;q32), t(4;14)(p16;q32), t(14;16)(q32;q23) were purchased from Cytocell. Nuclei were analyzed using an Olympus BX41
fluorescent light microscope. Two hundreds BMPC nuclei from each sample were scored. Cut-off levels for positive values represent the mean plus three SDs of abnormal cells from 15 healthy donors’ BMPC samples, and were adjusted to 15% for IgH translocations and 10% for deletions/gains. Chr 1 patterns were considered positive or negative as shown in Fig.1, panel C and D.

Statistical Analysis

Primary end points were overall survival (OS), defined as the time from study entry to death from any cause, and progression-free survival (PFS), defined as the time from study entry until documented progression or death for MM. Patients still alive and progression-free were censored at the date of last contact.

For univariate analyses, OS and PFS curves were estimated by the Kaplan-Meier method and compared using the log-rank test. OS and PFS were also analyzed by the Cox proportional hazard model comparing, by the Wald test, chemotherapy (VMPT-VT vs VMP), age at diagnosis (>75 vs ≤75 yrs), ISS score (IIIvsIIvsI), abnormal chr1 [del(1p) and/or gain(1q)], del(13), del(17p), t(11;14), [t(4;14) and/or t(14;16)] (any vs none), CD19, CD20, CD45, CD56 and CD117 expression on ≥30% vs <30% of total PC and CD19+/CD117- combination (any vs none). The effect of the same risk factors on OS was assessed by the multivariate Cox model. A multivariate binary logistic regression model was used to test age, ISS, iFISH abnormalities (independent variables) as risk factors for the onset of abnormal chr1 (dependent variable).

Patient characteristics were tested using the Fisher’ exact test for categorical variables and the Mann-Whitney test for continuous ones. All reported p-values were two-sided, at the conventional 5% significance level. Data were analyzed as of April 2014 by SPSS 21.0.0 and R 2.15.2 software.
Results

Baseline characteristics of enrolled patients (N=511) have been described in a previous report. At the current median follow-up of 54 months from the start of therapy (range: 1 to 80), median PFS is 25 months and median OS is not reached yet (50.6%).

Chr1 iFISH analysis was performed in 278 patients, based on sample availability. These patients showed the same baseline characteristics as those in whom chr1 abnormalities were not analyzed (Table X, supplemental material).

The frequencies of del(13), del(17p), t(11;14), t(4;14), t(14;16) and high-risk group were previously reported by Palumbo et al. and are summarized in Table 1 together with chr1 abnormalities.

A higher frequency of abnormal chr1 patients was observed in the VMP group in comparison to the VMPT-VT; this is due to gain(1q) asymmetric distribution between the two arms, whereas del(1p) was equally distributed, (Table 1).

A Multivariate Logistic Regression Model, in order to identify protective/risk factors for the presence of an abnormal chr1 was performed including age, ISS and iFISH chromosomal abnormalities (del(13), del(17p), t(11;14) and t(4;14)/t(14;16)), (Table 2, panel A). Del(13) and t(4;14)/t(14;16) represent borderline independent risk factors, (OR, 1.80; 95%CI, 0.95-3.43; p=0.074 and OR, 2.06; 95%CI, 1.00-4.27; p=0.051, respectively) while t(11;14) shows a strong protective role (OR, 0.15; 95%CI, 0.05-0.47; p=0.001). Immunophenotypic features were also tested by logistic regression analysis, but they did not show any significant result (data not shown).

Chr1 association with cytogenetic and immunophenotypic features were displayed in Table Z and Figure 2 of the supplemental file.

Figure 1 shows Kaplan-Meier curves for PFS and OS according to abnormal chr1 status, underlying its significant negative impact on PFS (p=0.009). Abnormal chr1 shows an unusual trend on OS, because its presence seems to exercise a variable impact during time; this behaviour suggests that this cytogenetic feature should be considered as a time-dependent variable. This hypothesis has been
confirmed by the Shoenfeld test and, subsequently, Cox analyses were carried-out with time-
dependent methodology.

Del(13), del(17p), IgH translocations and high-risk cytogenetic group did not significantly affect OS 
or PFS of enrolled patients (data not shown), except for t(11;14) which displayed a borderline 
protective role on OS (HR, 0.35; 95% CI, 0.12-1.02; p=0.053).

BMPC immunophenotypic features are shown in Table Y (supplemental material) and were equally 
distributed between the two therapeutic arms. Expression of CD19, CD20, CD45, CD56, CD117 and 
cytoplasmic k or λ Ig-light-chains did not significantly influence OS or PFS (data not shown). 
Interestingly, by the analysis of several antigen combinations, we identified CD19+/CD117- patients 
as a particular risk category for OS, (HR, 3.51; 95% CI, 1.20-10.31; p=0.022,) but not for PFS. This 
class was present in 10.3% of all patients and was equally distributed between VMP and VMPT-VT 
group (9.9% vs 10.6%; p=0.871).

Based on univariate Cox analyses, a multivariate Cox regression model was tested for OS, including 
chemotherapy, age, ISS, abnormal chr1 and CD19+/CD117- phenotype (Table 2, panel B). 
Independent predictors for a worse OS were age (HR, 1.73; 95% CI, 1.01 to 2.98; p=0.047), 
abnormal chr1 (HR, 4.01; 95% CI, 1.35 to 11.94; p=0.012) and CD19+/CD117- immunophenotypic 
profile (HR, 2.62; 95% CI, 1.23 to 5.58; p=0.012).

**Differential effect of Thalidomide**

Taking into account that chr1 abnormalities were not equally distributed between the two therapeutic 
arms, abnormal chr1 and all the other variables were also separately analysed, in order to test the 
potential adverse interaction with T regimen (Table 3). Abnormal chr1 shows a significant adverse 
impact in VMPT-VT arm (HR, 3.08; 95% CI, 1.04-9.14; p=0.042), not confirmed in VMP arm (HR, 
2.61; 95% CI, 0.83-8.20; p=0.102). Moreover, our data suggest that T impairs survival in patients 
carrying del(17p) (VMPT-VT arm: HR, 4.28; 95% CI, 1.59-11.54; p=0.004 / VMP arm: HR, 1.11;
95% CI, 0.34-3.66; p=0.866). Conversely, the protective role of T has been highlighted in CD19+ (VMP arm: HR, 3.89; 95% CI, 1.13-13.38; p=0.031 / VMPT-VT arm: HR, 1.46; 95% CI, 0.29-7.44; p=0.649) and in CD19+/CD117- patients (VMP arm: HR, 5.00; 95% CI, 1.15-21.78; p=0.032 / VMPT-VT arm: HR, 2.33; 95% CI, 0.44-12.32; p=0.320) and advanced ISS stage (ISS III vs I in VMP arm: HR, 2.56; 95% CI, 1.36-4.82; p=0.004 / ISS III vs I in VMPT-VT arm: HR, 1.65; 95% CI, 0.86-3.14; p=0.129). Age significantly affects OS in both arms (VMP arm: HR, 2.29; 95% CI, 1.04-5.04; p=0.040 / VMPT-VT arm: HR, 4.53; 95% CI, 1.84-11.14; p=0.001). No significant difference was highlighted for the other cytogenetic abnormalities or immunophenotypic features between the two arms.
Discussion

The introduction of novel agents in MM clinical practice highlights the need for new risk predictors and, although cytogenetic abnormalities represent strong prognostic factors, their real role is still a matter of debate.

Del(13), del(17p), IgH translocations and the high-risk group did not show a significant impact on OS and PFS of patients enrolled in VMP vs VMPT-VT trial. This finding confirms and emphasizes the already reported beneficial role of V, which seems to overcome the negative impact of poor prognostic cytogenetic features. This was demonstrated not just in the study by Palumbo et al., but also in the Harousseau et al. bortezomib-based trial, showing a similar PFS between high-risk and standard-risk patients. Moreover, the Spanish VISTA trial, comparing MP and VMP, showed that, in VMP subgroup, there was no statistically significant difference between high-risk and standard-risk patients in terms of OS. In line with all these findings, in our patients series, del(17p), t(4;14) and t(14;16) do not have any impact on clinical outcome, also at the present follow-up. Furthermore, a very recent paper from the Mayo Clinic set the new guidelines for MM treatment defining that: (1) t(4;14) patients should receive V as part of induction and maintenance treatment for at least one year, in order to overcome t(4;14) adverse impact on OS; (2) high-risk patients should receive lenalidomide, V and dexamethasone; (3) standard-risk patients can be treated with low-toxicity regimens involving lenalidomide and low dose dexamethasone.

The clinical impact of chr1 abnormalities has been so far evaluated in heterogeneous groups of MM patients, treated with different therapeutic regimens and gain(1q) and del(1p) were considered so closely related that it is hard to determine their distinct clinical impact. In this study we referred only to “abnormal chr1”, defined as del(1p) and/or gain (1q), which was present in 50.7% of patients and its poor prognostic impact on OS and PFS was more significant than that of del(1p) or gain(1q) considered separately (data not shown).
The logistic regression analysis identified del(13) and t(4;14)/t(14;16) as borderline risk factors for the presence of abnormal chr1, while t(11;14) emerged as a strong protective factor. These data (Table 2, panel A) did not describe only an association, but they highlighted a cause-effect relationship between the presence/absence of some chromosomal abnormalities and the onset of an abnormal chr1.

Del(1p) was equally distributed between the two arms, while a higher frequency of gain(1q) was observed in the VMP group as compared to the VMPT-VT. This bias may occur since patients were not randomized in the light of cytogenetic characteristics. Chr1 abnormalities were more frequent in VMP arm and we expected to observe a major negative survival impact in this group. Interestingly, a significant negative impact on OS was contrarily observed in VMPT-VT arm only, as shown in table 3. In other words, although abnormal chr1 was less frequent in VMPT-VT arm, its negative impact was significant just in this subgroup, probably due to a negative effect of T administration on these patients.

Smetana et al. analyzed several chromosomal abnormalities in 102 relapsed patients treated with V or T-based regimens. They suggest that V should be preferred to T in patients with relapsed and/or refractory MM carrying gain(1q), two or more cytogenetic abnormalities and/or del(17p).

Our findings show the ability of VMP treatment to overcome the negative prognostic impact of abnormal chr1 in newly diagnosed elderly patients, whereas the addiction of T appears to play a negative role on OS. Recently, the MRC Myeloma IX trial examined the role of T both as induction and maintenance therapy in del(17p) patients (n=85). T induction was associated with improved response rates, but not with improved OS, while, as maintenance therapy, it was associated with impaired survival. In our cohort of del(17p) patients (n=55), T impaired OS as shown by the univariate Cox analyses in Table 3. Moreover, also Kaplan-Meier analyses highlighted a negative effect of T on del(17p) patients (13.5 months in VMPT vs 22.5 months in VMP arm, p=0.726), even though not statistically significant, probably due to the low frequency of del(17p) (14.6%).
whereas in patients characterized by a normal(17p), the benefit of T has been observed (42.3 months in VMPT vs 31.7 months in VMP, p=0.061). The same trend was also confirmed by Kaplan-Meier analysis for PFS (del(17p) patients: 16.8 months in VMPT vs 19.5 months in VMP arm, p=0.329; norm(17p) patients: 34.5 months in VMPT vs 23.0 months in VMP, p<0.001). In our study, we could not distinguish between the effects of T as induction or maintenance therapy, because all the patients in the VMPT arm also received VT maintenance, whereas patients in VMP did not receive T at all. The detrimental role of T on OS was also evaluated in the whole patient series of the MRC Myeloma IX trial by Brioli et al.\textsuperscript{40}, who underlined its negative effect on high-risk patients. Some authors suggest, instead, that T maintenance is more beneficial in high-risk disease.\textsuperscript{41} We did not observe any significant difference comparing the high-risk and the standard-risk groups, confirming the benefit of V administration in high-risk patients independently from T administration.

The prognostic impact of PC immunophenotypic features has been largely investigated by several authors.\textsuperscript{3,4,7} More recently, CD19 expression on MM PC has been deeply discussed, underlying its role as an adverse prognostic marker\textsuperscript{5,7}, while CD117 was found to be associated with a favourable outcome.\textsuperscript{3} In our study, we did not observe any association between clinical outcome and the single expression of CD45, CD20, CD117, CD56. Mateo et al.\textsuperscript{3} published an extensive study on 685 newly diagnosed MM patients from GEM 2000 protocol. Their results outlined the prognostic relevance of three individual markers: CD19, CD28 and CD117. We observed that CD19+/CD117- patients were characterized by a shorter OS, but not PFS. When the analysis was carried out in the two therapeutic arms separately, the poor influence of this antigen combination emerged in the VMP arm only, suggesting that T administration may overcome its adverse impact.

Recently, it has been argued that the prognostic impact of genetic lesions is modulated over time by changes in the myeloma microenvironment and/or by the interaction with newborn cytogenetic abnormalities\textsuperscript{42}. For instance, MAF translocations [including t(14;16) and t(14;20)] are associated
with a poor prognosis in MM whereas the t(14;20) was not linked to disease progression in MGUS/smouldering MM patients.\textsuperscript{43,44} Furthermore, time dependency of prognostic features was also highlighted by Barlogie et al.\textsuperscript{45-47} In recent years, survival of MM patients has been extended from 5 to 10 years or more, as a result of autotransplant-supported high-dose M treatment.\textsuperscript{48,49} This longer follow-up leads to biphasic or triphasic patterns in Kaplan-Meier curves, suggesting that several parameters might govern different time segments of survival outcomes.\textsuperscript{46} Cytogenetic abnormalities detected by gene expression profiling\textsuperscript{45}, LDH and calcium levels\textsuperscript{46}, as well as complete response\textsuperscript{47} have been already described as time-dependent variables. These observations support our findings concerning the time-dependent effect of abnormal chr1 detected by iFISH, and CD19+/CD117- BMPC.

In the multivariate Cox analysis on the whole patient series, T protective role was not confirmed, while age>75, abnormal chr1 and CD19+/CD117- expression were independent predictors for OS.

In summary, our findings suggest that abnormal chr1 is an adverse prognostic factors, for OS and PFS, in elderly MM patients enrolled in the GIMEMA-MM-03-05 trial. CD119+/CD117- BMPC immunophenotype has an adverse impact too on OS; however, this antigen combination is rare, respect to abnormal chr1, which represents a large cluster with a major impact on OS, as revealed by the Cox multivariate analysis. Other genetic abnormalities do not have any impact on OS or PFS, probably due to V-administration. However, T-administration, even when associated with V, seems to have a negative effect on abnormal chr1 as well as del(17p) patients, whereas was of benefit in CD19+/CD117- and in advanced ISS patients.

Our study is a retrospective and explorative study aimed at better understanding the effect of abnormal chr1 on elderly MM patients treated with novel agents and our results highlight a complex picture of multiple interactions among therapy, risk predictors and time. Although these results need to be confirmed in prospective and larger studies, they may help designing future clinic trials.
Authorship and Disclosures

Contribution: All authors provided substantial contributions in study conception and in the acquisition, analysis or interpretation of data. They also revised the article at any stage and finally approved the version to be published. S.C., M.R., S.A., M.G. and D.O. mainly performed the laboratory analyses (multiparameter flow cytometry, plasma cells purification, interphase fluorescence in situ hybridization). S.C. and M.R. mainly designed the FISH and immunophenotypic study, collected/merged/analyzed all the data and wrote the manuscript. P.O. supervised the project. R.P. performed statistical analyses. F.G., S.B., C.M., L.B., P.M., M.T.P., G.G., B.B., A.P., M.B. were involved in the trial design, patients enrolment and clinical data collection and analysis.

Conflict-of-interest disclosure: S.C., M.R., S.A., M.G., D.O., C.M., L.B., R.P., B.B., P.O. declare no conflict of interest. F.G. has received honoraria from Celgene and Janssen-Cilag; advisory committee for Celgene and Byotest. S.B. has received honoraria from Celgene, Janssen-Cilag and Novartis; advisory committee for Merck Sharp & Dohme; consultancy from Onyx. P.M. has received honoraria and research funding from Celgene and Janssen and he also has consulted for Celgene. M.T.P. has received honoraria from Celgene and Janssen-Cilag. G.G. has consulted in Advisory Boards for Celgene. A.P. has received honoraria and he also has consulted for Amgen, Bristol-Myers Squibb, Celgene, Janssen, Millenium, Onyx. M.B. has received research support, consultancy and scientific advisory board from Celgene and Janssen Cilag.
References


38. Chang H, Qi X, Jiang A, Xu W, Young T, Reece D. 1p21 deletions are strongly associated with 1q21 gains and are an independent adverse prognostic factor for the outcome of high-dose chemotherapy in patients with multiple myeloma. Bone Marrow Transplant. 2010;45(1):117-21.


**Tables and Figures**

CONSORT diagram relative to the VMP vs VMPT-VT trial was previously published.\textsuperscript{31,32}

**Table 1.** Baseline frequency of chromosomal abnormalities in BMPC detected by iFISH.

<table>
<thead>
<tr>
<th>CHROMOSOMAL ABNORMALITIES</th>
<th>VMP</th>
<th>VMPT-VT</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>del(13)</td>
<td>101/192 (52.6%)</td>
<td>86/184 (46.7%)</td>
<td>0.569</td>
</tr>
<tr>
<td>del(17p)</td>
<td>32/192 (16.7%)</td>
<td>23/184 (12.5%)</td>
<td>0.399</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>31/192 (16.1%)</td>
<td>20/184 (10.9%)</td>
<td>0.248</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>33/192 (17.2%)</td>
<td>26/184 (14.1%)</td>
<td>0.579</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>9/192 (4.7%)</td>
<td>6/184 (3.3%)</td>
<td>0.678</td>
</tr>
<tr>
<td>high-risk</td>
<td>51/186 (27.4%)</td>
<td>65/194 (33.5%)</td>
<td>0.221</td>
</tr>
<tr>
<td>del(1p)</td>
<td>15/131 (11.4%)</td>
<td>9/147 (6.1%)</td>
<td>0.139</td>
</tr>
<tr>
<td>gain(1q)</td>
<td>74/131 (56.5%)</td>
<td>57/147 (38.8%)</td>
<td>0.005</td>
</tr>
<tr>
<td>abnormal Chr1 [del(1p) and/or gain(1q)]</td>
<td>79/131 (60.3%)</td>
<td>62/147 (42.2%)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Abbreviations: BMPC=bone marrow plasma cells, iFISH=interphase fluorescence in situ hybridization, VMP=Bortezomib Melphalan Prednisone, VMPT-VT=Bortezomib Melphalan Prednisone and Thalidomide followed by Bortezomib and Thalidomide maintenance, del=deletion, t=translocation, Chr=chromosome, high-risk=patients carrying del(17p) and/or t(4;14) and/or t(14;16).
### Table 2. Multivariate Regression Models.

#### Panel A: multivariate logistic regression model for abnormal chr1

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age&gt;75 years</td>
<td>1.26</td>
<td>0.60-2.65</td>
<td>0.537</td>
</tr>
<tr>
<td>ISS III vs II vs I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISS II vs I</td>
<td>1.22</td>
<td>0.55-2.70</td>
<td>0.631</td>
</tr>
<tr>
<td>ISS III vs I</td>
<td>1.38</td>
<td>0.56-3.39</td>
<td>0.483</td>
</tr>
<tr>
<td>Del(13)</td>
<td>1.80</td>
<td>0.95-3.43</td>
<td>0.074</td>
</tr>
<tr>
<td>Del(17p)</td>
<td>0.62</td>
<td>0.26-1.46</td>
<td>0.273</td>
</tr>
<tr>
<td>(11;14)</td>
<td>0.15</td>
<td>0.05-0.47</td>
<td>0.001</td>
</tr>
<tr>
<td>(t(4;14)/(t(14;16)</td>
<td>2.06</td>
<td>1.00-4.27</td>
<td>0.051</td>
</tr>
</tbody>
</table>

#### Panel B: multivariate Cox regression model for OS

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VMPT-VT vs VMP</td>
<td>0.94</td>
<td>0.35-2.55</td>
<td>0.906</td>
</tr>
<tr>
<td>Age&gt;75 years</td>
<td>1.73</td>
<td>1.01-2.98</td>
<td>0.047</td>
</tr>
<tr>
<td>ISS III vs II vs I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISS II vs I</td>
<td>1.80</td>
<td>0.92-3.51</td>
<td>0.086</td>
</tr>
<tr>
<td>ISS III vs I</td>
<td>1.93</td>
<td>0.85-4.37</td>
<td>0.115</td>
</tr>
<tr>
<td>abnormal chr1*</td>
<td>4.01</td>
<td>1.35-11.94</td>
<td>0.012</td>
</tr>
<tr>
<td>CD19+/CD117-*</td>
<td>2.62</td>
<td>1.23-5.58</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Abbreviations: Chr=Chromosome, OR=Odds Ratio, CI=Confidence Interval, HR=Hazard Ratio, OS=Overall Survival, VMP=Bortezomib Melphalan Prednisone, VMPT-VT= Bortezomib Melphalan Prednisone and Thalidomide followed by Bortezomib and Thalidomide maintenance, ISS=international staging system, del=deletion, t=translocation, Chr=chromosome, (t(4;14)/(t(14;16)= patients carrying t(4,14) and/or t(14,16), abnormal chr1= patients carrying del(1p) and/or gain(1q), CD19+/CD117-= patients carrying plasma cells CD19+ and CD117-, *=treated as time-dependent variable.
Table 3. Univariate Cox analyses for OS: impact of baseline clinical and biological characteristics in VMP or VMPT-VT arm.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>VMP</th>
<th></th>
<th></th>
<th>VMPT-VT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95%CI</td>
<td>p-value</td>
<td>HR</td>
<td>95%CI</td>
<td>p-value</td>
</tr>
<tr>
<td>Age &gt;75 years</td>
<td>2.29</td>
<td>1.04-5.04</td>
<td>0.040</td>
<td>4.53</td>
<td>1.84-11.14</td>
<td>0.001</td>
</tr>
<tr>
<td>ISS III vs II vs I</td>
<td></td>
<td></td>
<td>0.006</td>
<td></td>
<td></td>
<td>0.23</td>
</tr>
<tr>
<td>ISS II vs I</td>
<td>2.44</td>
<td>1.37-4.36</td>
<td>0.003</td>
<td>1.06</td>
<td>0.59-1.91</td>
<td>0.834</td>
</tr>
<tr>
<td>ISS III vs I</td>
<td>2.56</td>
<td>1.36-4.82</td>
<td>0.004</td>
<td>1.65</td>
<td>0.86-3.14</td>
<td>0.129</td>
</tr>
<tr>
<td>del(13)</td>
<td>1.22</td>
<td>0.51-2.91</td>
<td>0.653</td>
<td>2.48</td>
<td>0.94-6.51</td>
<td>0.065</td>
</tr>
<tr>
<td>del(17p)</td>
<td>1.11</td>
<td>0.34-3.66</td>
<td>0.866</td>
<td>4.28</td>
<td>1.59-11.54</td>
<td>0.004</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>0.28</td>
<td>0.05-1.57</td>
<td>0.147</td>
<td>0.41</td>
<td>0.10-1.60</td>
<td>0.199</td>
</tr>
<tr>
<td>t(4;14)/ t(14;16)</td>
<td>0.78</td>
<td>0.30-2.01</td>
<td>0.606</td>
<td>1.08</td>
<td>0.35-3.28</td>
<td>0.894</td>
</tr>
<tr>
<td>abnormal chr1*</td>
<td>2.61</td>
<td>0.83-8.20</td>
<td>0.102</td>
<td>3.08</td>
<td>1.04-9.14</td>
<td>0.042</td>
</tr>
<tr>
<td>high-risk</td>
<td>1.02</td>
<td>0.39-2.65</td>
<td>0.966</td>
<td>2.20</td>
<td>0.87-5.52</td>
<td>0.094</td>
</tr>
<tr>
<td>CD19+/CD117-</td>
<td>5.00</td>
<td>1.15-21.78</td>
<td>0.032</td>
<td>2.33</td>
<td>0.44-12.32</td>
<td>0.320</td>
</tr>
</tbody>
</table>

Abbreviations: OS=Overall Survival, VMP=Bortezomib Melphalan Prednisone, VMPT-VT= Bortezomib Melphalan Prednisone and Thalidomide followed by Bortezomib and Thalidomide maintenance, CI=Confidence Interval, HR=Hazard Ratio, del=deletion, t=translocation, Chr=chromosome, ISS=international staging system, t(4;14)/t(14;16)= patients carrying t(4;14) and/or t(14;16), abnormal chr1= patients carrying del(1p) and/or gain(1q), high-risk= patients carrying del(17p) and/or t(4;14) and/or t(14;16), CD19+/CD117-= patients carrying plasma cells CD19+ and CD117-, *=treated as time-dependent variable
Figure Legend

Figure 1. Abnormal Chr1: Kaplan Meier curves and iFISH patterns. Clinical outcome of patients carrying abnormal chr1 [del(1p) and/or gain(1q)]: (A) Kaplan Meier curve for PFS; (B) Kaplan Meier curve for OS; (C) iFISH patterns for del(1p): 2 green signals in plasma cells with normal 1p and 1 green signal in plasma cells carrying 1p deletion; (D) iFISH patterns for gain(1q): 2 red signals in plasma cells with normal 1q and 3 or more signals in plasma cells carrying 1q gain. (Images captured with a Duet System, BioView Ltd, Israel)