

Genetic abnormalities and survival in multiple myeloma: the experience of the Intergrupe Francophone du Myélome.

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Supported in part by grants from the Association pour la Recherche sur le Cancer, from the Ligue contre le Cancer (Equipe Labélisée), and from the French Ministry of Health (PHRC 2002).

We are indebted to Mrs Marine Aliaga, Nadège Gouy, Marie-Christine Boussier, and Karine Pennarun for excellent technical expertise.

H Avet-Loiseau designed and performed the research, and wrote the paper, M Attal, P Moreau, and F Garban designed the research and coordinated one of the clinical trials, C Charbonnel performed the statistical analysis, and all the other co-authors are members of the IFM board, and did participate to the design of the research and to the patients management.

Abstract

Acquired genomic aberrations have been shown to significantly impact survival in several hematological malignancies. We analyzed the prognostic value of the most frequent chromosomal changes in a large series of patients with newly diagnosed symptomatic myeloma prospectively enrolled in homogeneous therapeutic trials. All the 1064 patients enrolled in the IFM99 trials conducted by the Intergroupe Francophone du Myélome benefited from an interphase fluorescence in situ hybridization analysis performed on purified bone marrow plasma cells. They were systematically screened for the following genomic aberrations: del(13), t(11;14), t(4;14), hyperdiploidy, *MYC* translocations, and del(17p). Chromosomal changes were observed in 90% of the patients. The del(13), t(11;14), t(4;14), hyperdiploidy, *MYC* translocations, and del(17p) were present in 48%, 21%, 14%, 39%, 13%, and 11% of the patients, respectively. After a median follow-up of 41 months, univariate statistical analyses revealed that del(13), t(4;14), non-hyperdiploidy, and del(17p) negatively impacted both the event free survival and the overall survival, whereas t(11;14) and *MYC* translocations did not influence the prognosis. Multivariate analyses on 513 patients annotated for all the parameters showed that only t(4;14) and del(17p) retained prognostic value for both the event free and overall survivals. When compared with the currently used International Staging System, this prognostic model compares favorably. In myeloma, the genomic aberrations t(4;14) and del(17p), together with β 2-microglobulin level, are important independent predictors of survival. These findings have implications for the design of risk-adapted treatment strategies.

Multiple myeloma is the second most common hematological cancer, representing one percent of all cancer diagnoses and two percent of all cancer deaths. Despite recent progress in the management of patients, myeloma remains an incurable disease, with a median survival not exceeding 4 years.¹ However, this uniform evolution hides a wide heterogeneity in clinical course, some patients dying from disease evolution within a few weeks, whereas others live for more than 10 years. Several prognostic staging systems have been proposed, the most powerful being the recently reported International Staging System (ISS), based on the evaluation of two simple biological parameters, i.e., the serum levels of β 2-microglobulin and albumin.² Nevertheless, this staging system did not really include the role of (cyto)genetics since very few patients were analyzed for this parameter.

Chromosomal abnormalities have been shown to display a major role in disease evolution in several hematological malignancies. In myeloma, cytogenetics has been hampered by the low proliferative activity of the malignant plasma cells in vitro, and by the frequent low tumor cell infiltrate within the bone marrow specimens. Most large series reported about 30% abnormal karyotypes,³⁻⁵ although other techniques not dependent upon metaphase obtaining described genomic aberrations in almost 100% of the cases.^{6,7} Fluorescence in situ hybridization (FISH) allows to circumvent this pitfall, since it enables the detection of specific chromosomal changes even in non-cycling interphase cells. Initial studies using this technique in myeloma demonstrated a high incidence of chromosomal changes,^{7,8} and suggested that FISH could be used for the assessment of single abnormalities useful for prognostic evaluation.⁹⁻¹³

We designed a study based on interphase FISH for the evaluation of a large series of homogeneously treated patients, using DNA probes specific for the most recurrent chromosomal aberrations observed in myeloma. Our objective was to assess the incidence and clinical relevance of genomic abnormalities in this group of patients treated with high-dose therapy.

METHODS

Approval for this study has been obtained from the University Hospitals of Nantes, Toulouse and Genoble institutional review boards. Informed consent was provided according to the Declaration of Helsinki.

Patients

Between April 2000 and December 2003, 1064 patients under 66 years of age with symptomatic newly-diagnosed multiple myeloma were enrolled in the IFM99 therapeutic trials, run by the Intergroupe Francophone du Myélome (IFM), (for 81 patients, bone marrow has been analyzed in other laboratories). Briefly, patients received an induction therapy with 4 courses of VAD (vincristine, adriamycin and dexamethasone), followed by double intensive therapy. The IFM99-02 trial was dedicated for patients with less than 2 poor-prognosis factors (β 2-microglobulin >3 mg/l, del(13) by FISH). After induction, patients received 2 courses of high-dose melphalan (140 mg/m² and 200 mg/m²), and were then randomized for maintenance therapy: none (arm A), pamidronate (arm B), or pamidronate + thalidomide (arm C) until relapse. This trial recruited 780 patients.¹⁴ The IFM99-03 trial enrolled 65 patients with 2 poor-prognosis factors and with an HLA-identical familial donor.¹⁵ After induction, patients received one high-dose melphalan course (200 mg/m²), followed by a reduced intensity conditioned allogeneic transplant. Finally, the IFM99-04 trial enrolled 219 patients with 2 poor-prognosis factors and no HLA-identical familial donor.¹⁶ After a similar induction and first high-dose melphalan course, patients received a second melphalan-based intensification (220 mg/m²), and were randomized to receive or not an anti-IL6 antibody during the conditioning regimen. All the patients were analyzed for del(13) by FISH on bone marrow at diagnosis, and 983 of them were referred to the Hematology laboratory of Nantes and are reported in this study.

Interphase cytogenetics analysis

After overnight shipment, mononuclear cells were separated by gradient-density centrifugation (Ficoll-Hypaque, Eurobio, Les Ulis, France). Plasma cells were then purified using CD138-coated magnetic beads according to the manufacturer's instructions (Miltenyi Biotec, Paris, France), enabling a plasma cell purity higher than 90% (controlled in each patient), as previously described.⁸ Plasma cells were then analyzed using DNA probes specific for the following chromosomal aberrations: del(13q14), t(11;14)(q13;q32), t(4;14)(p16;q32), *MYC* rearrangements, hyperdiploidy, and del(17p13). The del(13) was analyzed with a probe specific for the D13S319 locus (purchased from Abbott, Rungis, France). Probes specific for the t(4;14) and t(11;14) translocations were kindly provided by Abbott (Chicago, IL). Hyperdiploidy was assessed using a set of probes specific for chromosomes 5, 9 and 15 (kindly provided by Abbott, Chicago, IL), as previously described (hyperdiploidy if at least 2 probes show extracopies).¹⁷ Translocations involving the *MYC*

locus were detected using YAC probes previously described,¹⁸ and a commercially available probe, kindly provided by Abbott. The del(17p) was assessed using a *P53*-specific BAC probe at 17p13 (RPCI-613o12). Probe labeling and FISH procedures have been previously described.⁸

Statistical analyses

The primary end point was the correlation with survival from the time of diagnosis. Kaplan-Meier curves for event-free survival (EFS, defined by the time between diagnosis and the occurrence of progression, relapse, or death) and overall survival (OS) were plotted and compared by the use of the log-rank test. Comparison of frequencies between groups was performed using the chi-2 test. Prognostic factors for event-free survival and overall survival were determined by means of the Cox proportional hazard model for covariate analysis. As possible prognostic factors, β 2-microglobulin levels, albumin levels, hemoglobin levels, platelet counts, isotype, and presence or absence of genomic aberrations [del(13), t(11;14), t(4;14), *MYC* translocations, hyperdiploidy, del(17p)] were included in the regression model. For continuous variables, classical cut-offs were selected. To take into account the possible effects of treatment upon prognostic variables, treatment-adjusted statistical analyses were performed. The statistical analyses were performed with the SAS 9.1 software package (SAS Institute Inc., Cary, NC).

RESULTS

Interphase cytogenetic analysis

Since del(13) assessment was required for enrolment in the different trials, patients were primarily analyzed for 13q deletions. Among the 983 bone marrow aspirates received in the lab, 936 were assessable for del(13) (lack of plasma cells or FISH failure in 47 cases). Other probes were analyzed in the following order: t(11;14), t(4;14), hyperdiploidy, *MYC*, and del(17p). Because of the small number of purified plasma cells in many specimens (median % of plasma cells was 6%), these probes have been tested in 746, 716, 657, 571, and 532 patients, respectively (Table 1). Del(13) was observed in 449 patients (48%). The median % of plasma cells exhibiting del(13) was 70% (range 20-100). Translocation t(11;14) occurred in 154 patients (21%), and was associated with del(13) in 60 patients (39% of the t(11;14)-positive patients). Translocation t(4;14) was observed in 100 patients (14%), and was frequently associated with del(13) (85% of the t(4;14)-positive patients, $p=3.10^{-14}$). In contrast, t(4;14) and t(11;14) were never associated. Hyperdiploidy was assessed in 256 patients (39%), and 36% of them presented del(13). A lower incidence of t(11;14) and t(4;14) was also observed in patients with hyperdiploidy (2% and 4.6%, respectively). *MYC* translocations were observed in 74 patients (13%). No specific association was observed with other chromosomal abnormalities in these *MYC*-rearranged patients. Loss of 17p was present in 58 patients (11%), in a median of 75% of the plasma cells (range 32-94). Del(13) was detected in 78% of these patients ($p=5.10^{-5}$). Translocations t(4;14) and t(11;14) were seen in 11 and 6 patients with del(17p), respectively. No correlation was found with *MYC* rearrangements or ploidy.

Correlation with outcome

In a first step, we did analyze the prognostic impact of each individual chromosomal aberration on the EFS (Table 2 and Figure 1) and overall survival (Figure 2). When attained, the median survival is given in months from diagnosis. Conversely, if the median survival was not attained, the % of patients alive at the median follow-up (i.e., 41 months) are given. After a median follow-up of 41 months for surviving patients, 234 of the 936 patients analyzed for chromosomal abnormalities had died. The median EFS for patients with del(13), t(4;14), and del(17p) was 29 months (vs 41 months, $p=5.10^{-8}$), 20.6 months (vs 36.5 months, $p=1.10^{-12}$), and 15 months (vs 35 months, $p=5.10^{-11}$), respectively. Regarding OS, the median was attained for t(4;14) and del(17p), i.e., 41.3 months (vs 79% alive at 41 months, $p=2.10^{-8}$), and 22 months (vs 75% alive at 41 months, $p=4.10^{-12}$), respectively. For del(13), evaluation at 41 months showed a percentage of alive patients of 68% (vs 83%, $p=9.10^{-7}$). Regarding, t(11;14), hyperdiploidy, and *MYC* translocations, no (or marginal) impact on both EFS and OS was observed. For del(13) and del(17p), the prognostic impact was even greater if we split the patients presenting the deletions according to a cut-off of plasma cells presenting the abnormality. Serial analyses showed that the most powerful cut-offs were 74% for del(13) and 60% for del(17p). The median EFS were 27 months (vs 39 months, $p=3.10^{-9}$), and 14.6 months (vs 34.7 months, $p=5.10^{-11}$), respectively for patients with del(13) \geq 74% and del(17p) \geq 60%. Using these cut-offs, 59% of patients with del(13) \geq 74% were alive at 41 months (vs 80%, $p=2.10^{-7}$), whereas the median OS was 22.4 months for patients with del(17p) \geq 60% (vs 75% of patients alive if del(17p) $<$ 60%, $p=4.10^{-12}$).

Multivariate analysis

We then performed a multivariate analysis including all the chromosomal aberrations significantly associated with event free and overall survivals in the univariate analysis, i.e., del(13), t(4;14), del(17p), and ploidy, and other parameters shown to be associated with survival in this series: β 2-microglobulin level, albumin level, hemoglobin level, and platelet count (Table 3). The analysis was performed on the 513 patients for whom all the parameters were available. Four parameters were statistically independent predictors of EFS: t(4;14), del(17p), β 2-microglobulin, and hemoglobin level lower than 10 g/dl. A similar analysis for prediction of OS identified 3 factors: t(4;14), del(17p), and β 2-microglobulin, with the delineation of 3 groups of patients with highly divergent outcomes (Figure 3). Of note, these analyses showed that the prognostic value of del(13) was almost entirely dependent on the frequent association with t(4;14) and del(17p). In patients lacking these two abnormalities, del(13) is not anymore significant (Figure 4). Multivariate analyses clearly identified a group of patients with an excellent prognosis (36% of the series): those lacking t(4;14) and del(17p), with a low β 2-microglobulin level (expected survival at 4 years = 83%). Conversely, patients presenting either t(4;14) or del(17p), and a high β 2-microglobulin level have a median OS of only 19 months. Analyses according to treatment randomizations did not modify the results, these three parameters retaining their independent prognostic significance after treatment-adjusted analyses. We did also analyze the impact of these two chromosomal abnormalities in each ISS stage, in order to evaluate the possibility to improve survival prediction. We showed that t(4;14) and/or del(17p) separated two groups of patients within each ISS stage (Figures 5 and 6)

DISCUSSION

We found that even when focusing on a few number of recurrent chromosomal abnormalities, interphase FISH was able to detect genomic changes in almost 90% of the patients with myeloma at diagnosis, so about three times more frequently than conventional chromosomal banding. This striking difference is most likely due to the low number of plasma cells within the bone marrow specimens sent for laboratory purposes. In this series, the median percentage of plasma cells after mononuclear cell separation was only 6%. In addition, the usually low proliferative activity of malignant plasma cells, and the fact that some of the chromosomal changes are cytogenetically silent at karyotype (like t(4;14)), explain the low informativity of cytogenetics in myeloma. However, the low plasma cell infiltrate present in the samples imposes to identify the plasma cells before to perform interphase FISH. Despite these pitfalls, this study showed that genomic information might be obtained in at least 95% of the patients with myeloma, even in a multicenter setting.

This study is so far the largest series of patients with newly diagnosed myeloma, analyzed for genomic aberrations, enabling the description of definitive incidences of the most frequent chromosomal abnormalities. The del(13) is the most frequent abnormality (48%), followed by hyperdiploidy (39%), t(11;14) (21%), t(4;14) (14%), *MYC* translocations (13%), and del(17p) (11%). Moreover, all the patients have been treated with a homogeneous intensive strategy (double transplant in all the cases), allowing to perform highly valuable prognostic analyses. According to previously reported studies,⁹⁻¹¹ del(13) was predictive for both event free survival and overall survival, with highly significant p values. However, del(13) was not found to be an independent prognostic factor in the multivariate analysis. Actually, most of the prognostic power of del(13) was related to the t(4;14) and to the del(17p), frequently associated with del(13). In patients lacking t(4;14) and del(17p), del(13) was not anymore prognostic, whatever the cut-off chosen for its definition (Figure 4).

The analysis of t(4;14) and del(17p) was much more powerful in the prediction of both EFS and OS. Translocation t(4;14) was associated with a median EFS of 20.6 months and a median OS of 41.3 months, which are both highly significantly shorter than for patients lacking the translocation, in agreement with other smaller series.^{12,19-24} However, despite its high impact on survival (shown by the multivariate analysis), t(4;14) has to be evaluated in the context of other parameters, and especially β 2-microglobulin level. As shown in Figure 3, patients with a t(4;14) and a low β 2-microglobulin level displayed an outcome close to that of patients lacking the translocation but with a high β 2-microglobulin level. Similar comments can be made for del(17p).^{12,13,25} Patients presenting the deletion in more than 60% of their plasma cells had a short EFS (14.6 months) and OS (22.4 months), but patients with a low β 2-microglobulin level may expect a longer survival (Figure 3). The biological substratum of this major clinical impact is so far unknown, for both abnormalities. Translocation t(4;14) is known to deregulate two genes, *FGFR3* and *MMSET*.^{26,27} However, since *FGFR3* is not expressed in about one third of patients with t(4;14),^{20,21} the target gene is most likely *MMSET*, whose functions are currently not known. Similarly, the target gene(s) of del(17p) is (are) so far not identified. Even though several authors focused on the *P53* gene, formal demonstrations of its deregulation are currently lacking.

Other genomic changes have a lower impact on disease evolution. Translocation t(11;14) did not act upon survival, as suggested by some recent studies.^{28,29} The role of the consequently upregulated *CCND1* gene is not understood so far. Translocations involving *MYC* did not modify the course of the disease. However, as shown by gene profiling experiments,³⁰ *MYC* is activated in a large number of patients with myeloma, by other mechanisms than translocations, which may have hidden the prognostic impact of these rearrangements. Hyperdiploidy was marginally prognostic in this series. Previous series suggesting a favorable impact of hyperdiploidy on outcome were based on cytogenetics,^{5,31,32}

and thus restricted to patients with an informative karyotype, and were mostly retrospective series including non-homogeneously treated patients. Evaluation of ploidy by FISH was not dependent upon proliferation and may explore different groups of patients. Furthermore, the assessment of hyperdiploidy using FISH probably underestimates its frequency, and may slightly modify its specific prognostic impact. Nevertheless, because of the marginal impact of hyperdiploidy on survival, this pitfall did probably not introduce a major bias in the analysis. Regarding other potentially prognostic chromosomal aberrations not analyzed in this study, a specific comment is required for t(14;16)(q32;q23),^{33,34} which has been described as a poor-prognosis factor. Because of the scarcity of available plasma cells, we chose to focus our analysis on the most frequent genomic changes, and to not analyze t(14;16), present in less than 5% of the patients.⁸ This translocation being associated with a poor prognosis,¹² and being almost constantly associated with del(13), it is highly probable that it would have even “lightened” the prognostic impact of del(13). Finally, we showed that this prognostic model was independent of the treatment, at least in this series of young patients treated with tandem intensification. In particular, the poor prognosis associated with high β 2-microglobulin level, t(4;14) and del(17p) seemed to not be modified by the administration of thalidomide as maintenance therapy. However, because the IFM 99-02 trial was dedicated to patients with 0 or 1 poor-prognosis factor, these abnormalities were underrepresented in this trial. The analysis could only be performed for del(13), showing that these patients did not benefit from thalidomide maintenance.¹⁴ We then analyzed the role of cytogenetic abnormalities according to the International Staging System. In this classification, only a small minority of the patients were analyzed for cytogenetics and/or FISH. We show here that the genetic parameters highly improved the survival prediction power, in each ISS stage (Figure 6).

In conclusion, we show that genomic aberrations, evaluated by interphase FISH, play a major role in the evolution of patients with myeloma, extending previous conclusions focused on this topic.³⁵⁻³⁷ Analysis at diagnosis enables the identification of 3 groups in this large series of patients homogeneously treated by double transplants: patients who highly benefit from high-dose therapy (patients lacking t(4;14) and del(17p), and with a low β 2-microglobulin level), patients who have a short survival with this type of treatment (patients with either t(4;14) or del(17p), and a high β 2-microglobulin level), and an intermediate group. These analyses may have implications for the risk-adapted management of patients with myeloma, at least for the youngest ones. Whether these prognostic parameters are still valid in older patients, or in patients treated with other therapeutic strategies, remain opened questions, currently evaluated in other IFM trials.

References

- 1- Kyle RA, Rajkumar SV. Plasma cell disorders. In: Goldman L, Ausiello DA, eds. Cecil textbook of medicine. 22nd ed. Philadelphia: W.B. Saunders, 2004:1184-95.
- 2- Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. *J Clin Oncol* 2005;23:3412-20.
- 3- Dewald GW, Kyle RA, Hicks GA, Greipp PR. The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. *Blood* 1985;66:380-90.

- 4- Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic finding in 200 patients with multiple myeloma. *Cancer Genet Cytogenet* 1995;82:41-9.
- 5- Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart C. Hypodiploidy is a major prognostic factor in multiple myeloma. *Blood* 2001;98:2229-38.
- 6- Barlogie B, Alexanian R, Dixon D, Smith L, Smallwood L, Delasalle K. Prognostic implications of tumor cell DNA and RNA content in multiple myeloma. *Blood* 1985;66:338-41.
- 7- Drach J, Schuster J, Nowotny H, et al. Multiple myeloma: High incidence of chromosomal aneuploidy as detected by interphase fluorescence in situ hybridization. *Cancer Res* 1995;55:3854-9.
- 8- Avet-Loiseau H, Facon T, Grosbois B, et al. Oncogenesis of multiple myeloma: 14q32 and 13q chromosomal abnormalities are not randomly distributed, but correlate with natural history, immunological features and clinical presentation. *Blood* 2002;99:2185-91.
- 9- Zojer N, Königsberg R, Ackermann J, et al. Deletion of 13q14 remains an independent adverse prognostic variable in multiple myeloma despite its frequent detection by interphase fluorescence in situ hybridization. *Blood* 2000;95:1925-30.
- 10- Facon T, Avet-Loiseau H, Guillermin G, et al. Chromosome 13 abnormalities identified by FISH analysis and serum β 2-microglobulin produce a very powerful myeloma staging system for patients receiving high dose therapy. *Blood* 2001;97:1566-71.
- 11- Fonseca R, Harrington D, Oken MM, et al. Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (Δ 13) in multiple myeloma: An Eastern Cooperative Oncology Group study. *Cancer Res* 2002;62:715-20.
- 12- Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003; 101:4569-75.
- 13- Chang H, Qi C, Yi QL, Reece D, Stewart AK. p53 gene deletion detected by fluorescence in situ hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. *Blood*. 2005;105:358-60.
- 14- Attal M, Harousseau JL, Leyvraz S, et al. Maintenance treatment with thalidomide after autologous transplantation for myeloma: final analysis of a prospective randomized study of the IFM. *Blood* 2006, Epub ahead of print.
- 15- Garban F, Attal M, Michallet M, et al. Prospective comparison of autologous stem cell transplantation followed by a dose-reduced allograft (IFM99-03 trial) with tandem autologous stem cell transplantation (IFM99-04 trial) in high-risk de novo multiple myeloma. *Blood* 2006;107:3474-80.
- 16- Moreau P, Hulin C, Garban F, et al. Tandem autologous stem cell transplantation in high-risk de novo multiple myeloma: final results of the prospective and randomized IFM 99-04 protocol. *Blood*. 2006;107:397-403.
- 17- Wuilleme S, Robillard N, Lodé L, et al. Ploidy, as detected by fluorescence *in situ* hybridization, defines different subgroups in multiple myeloma. *Leukemia* 2005;19:275-8.
- 18- Avet-Loiseau H, Gerson F, Magrangeas F, et al. Rearrangements of the *c-myc* oncogene are observed in 15% of primary human multiple myeloma tumors. *Blood* 2001;98:3082-6.

- 19- Moreau P, Facon T, Leleu X, et al: Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. *Blood* 2002;100:1579-83.
- 20- Keats JJ, Reiman T, Maxwell CA, et al. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. *Blood* 2003;101:1520-9.
- 21- Santra M, Zhan F, Tian E, Barlogie B, Shaughnessy J. A subset of multiple myeloma harboring the t(4;14)(p16;q32) translocation lacks FGFR3 expression but maintains an *IGH/MMSET* fusion transcript. *Blood* 2003;101:2374-6.
- 22- Fonseca R, Debes-Marun CS, Picken EB, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. *Blood* 2003;102:2562-7.
- 23- Chang H, Sloan S, Li D, et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. *Br J Haematol* 2004;125:64-8.
- 24- Jaksic W, Trudel S, Chang H et al. Clinical outcomes in t(4;14) multiple myeloma: a chemotherapy-sensitive disease characterized by rapid relapse and alkylating agent resistance. *J Clin Oncol* 2005;23:7069-73.
- 25- Drach J, Ackermann J, Fritz E, et al. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. *Blood* 1998;92:802-9.
- 26- Chesi M, Nardini E, Brents LA, et al. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet* 1997;16:260-4.
- 27- Chesi M, Nardini E, Lim RS, et al. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, *MMSET*, resulting in *IgH/MMSET* hybrid transcripts. *Blood* 1998;92:3025-34.
- 28- Fonseca R, Blood EA, Oken MM, et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood* 2002;99:3735-41.
- 29- Stewart AK, Fonseca R. Prognostic and therapeutic significance of myeloma genetics and gene expression profiling. *J Clin Oncol* 2005;23:6339-44.
- 30- Zhan F, Hardin J, Kordsmeier B, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood* 2002;99:1745-57.
- 31- Fassas ABT, Spencer T, Sawyer J, et al. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. *Br J Haematol* 2002;118:1041-7.
- 32- Debes-Marun CS, Dewald GW, Bryant S, et al. Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. *Leukemia* 2003;17:427-36.
- 33- Chesi M, Bergsagel PL, Shonukan OO, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. *Blood* 1998;91:4457-63.
- 34- Hurt EM, Wiestner A, Rosenwald A, et al. Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. *Cancer Cell* 2004;5:191-9.

- 35- Mitsiades CS, Mitsiades N, Munshi NC, Anderson KC. Focus on multiple myeloma. *Cancer Cell* 2004;6:439-44.
- 36- Hideshima T, Bergsagel PL, Kuehl WM, Anderson K. Advances in biology of multiple myeloma: clinical applications. *Blood* 2004;104:607-18.
- 37- Bergsagel PL, Kuehl WM. Molecular pathogenesis and a consequent classification of multiple myeloma. *J Clin Oncol* 2005;23:6333-8.

Table 1: Incidence of chromosomal abnormalities in multiple myeloma.

Genomic Aberrations	Incidence (number of patients analyzed for the aberration)
Del(13)	48% (936)
t(11;14)(q13;q32)	21% (746)
t(4;14)(p16;q32)	14% (716)
Hyperdiploidy	39% (657)
<i>MYC</i> translocations	13% (571)
Del(17p)	11% (532)

Table 2: Prognostic value of chromosomal abnormalities (univariate analysis).

Genomic Aberrations	Impact on event free survival* (p value)	Impact on overall survival** (p value)
Del(13)	29 months vs 41 months (p=5.10 ⁻⁸)	68% vs 83% (p=9.10 ⁻⁷)
t(11;14)(q13;q32)	35 months vs 34 months (p=0.2)	80% vs 74% (p=0.28)
t(4;14)(p16;q32)	20.6 months vs 36.5 months (p=1.10 ⁻¹²)	41.3 months vs 79% (p=2.10 ⁻⁸)
Hyperdiploidy	37 months vs 33 months (p=0.02)	82% vs 70% (p=0.006)
<i>MYC</i> translocations	35 months vs 37 months (p=0.94)	72% vs 78% (p=0.50)
Del(17p)	15 months vs 35 months (p=5.10 ⁻¹¹)	22 months vs 75% (p=4.10 ⁻¹²)

* Median event free survival for patients presenting the chromosomal abnormality vs that of those who did not present the genomic aberration.

** Median overall survival for patients presenting the chromosomal abnormality vs that of those who did not present the genomic aberration. When the median was not attained, we did calculate the % of patients alive at the time of median follow-up, i.e., 41 months.

Table 3: Results of Cox regression analysis of event free survival and overall survival time from diagnosis.

Prognostic parameters*	Hazard ratio for event free survival (95% CI)	Hazard ratio for overall survival (95% CI)
Del(17p) > 60%	3.29 (2.23-4.87), p=2.10 ⁻⁹	3.93 (2.54-6.08), p=8.10 ⁻¹⁰
t(4;14)	2.79 (2.05-3.79), p=6.10 ⁻¹¹	2.78 (1.90-4.06), p=1.10 ⁻⁷
β2m > 4 mg/l	1.67 (1.28-2.18), p=0.0002	2.83 (2.02-3.97), p=2.10 ⁻⁹
Hb < 10 g/dl	1.38 (1.06-1.81), p=0.0174	

* β2m = β2-microglobulin level; Hb = Hemoglobin level.

Legends to Figures

Figure 1 : Impact of genomic aberrations on event free survival.

Panel A shows a Kaplan-Meier plot of the impact of del(13) on event free survival for the 936 patients analyzed for this abnormality. Panel B shows the impact of t(4;14), analyzed in 716 patients. Panel C shows the value of del(17p) on event free survival of 532 patients. The blue curve is for patients presenting the genomic abnormality, whereas the black curve represents the event free survival of patients lacking the chromosomal aberration.

Figure 2: Impact of genomic aberrations on overall survival.

Panel A shows a Kaplan-Meier plot of the impact of del(13) on overall survival for the 936 patients analyzed for this abnormality. Panel B shows the impact of t(4;14), analyzed in 716 patients. Panel C shows the value of del(17p) on overall survival of 532 patients. The blue curve is for patients presenting the genomic abnormality, whereas the black curve represents the event free survival of patients lacking the chromosomal aberration.

Figure 3: Influence of t(4;14), del(17p) and β2-microglobulin level on overall survival.

The black curve is for the 155 patients lacking del(13), t(4;14) and del(17p), and presenting a low β2-microglobulin level (≤ 4 mg/l). The green curve represents the same patients, but with a high β2-microglobulin level (> 4 mg/l) (74 patients). The blue curve depicts the 110 patients lacking t(4;14), and del(17p), with a low β2-microglobulin level, but presenting a del(13). The red curve represents the 69 patients lacking both t(4;14) and del(17p), with a high β2-microglobulin level, and with a del(13). The grey curve shows the 63 patients with either a t(4;14) or a del(17p) in more than 60% of their plasma cells, and a low β2-microglobulin level. Finally, the pink curve shows the overall survival of the 42 patients with either a t(4;14) or a del(17p) in more than 60% of their plasma cells, and a high β2-microglobulin level.

Figure 4: Prognostic impact of del(13) in patients lacking t(4;14) and del(17p).

Panel A depicts the prognostic influence of del(13) on event free survival in patients presenting neither t(4;14), nor del(17p), whereas panel B shows its impacts on overall survival. No statistically significant difference was observed for both event free survival (p=0.12), and overall survival (p=0.41).

Figure 5: Survival according the ISS stages

Panel A depicts event free survival according to the ISS stages and panel B shows overall survival (in months).

Figure 6: Kaplan-Meyer estimates of survival according to the ISS stages and t(4;14) and/or del(17p).

Panel A shows event free survival among patients in the various ISS stages according to the presence or not of t(4;14) and/or del(17p). Panel B shows overall survival (in months).

Figure 1

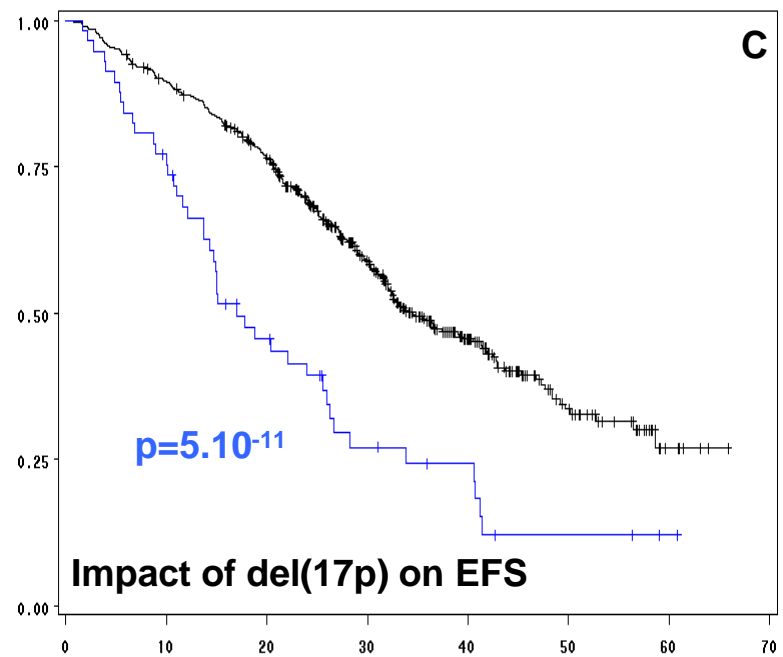
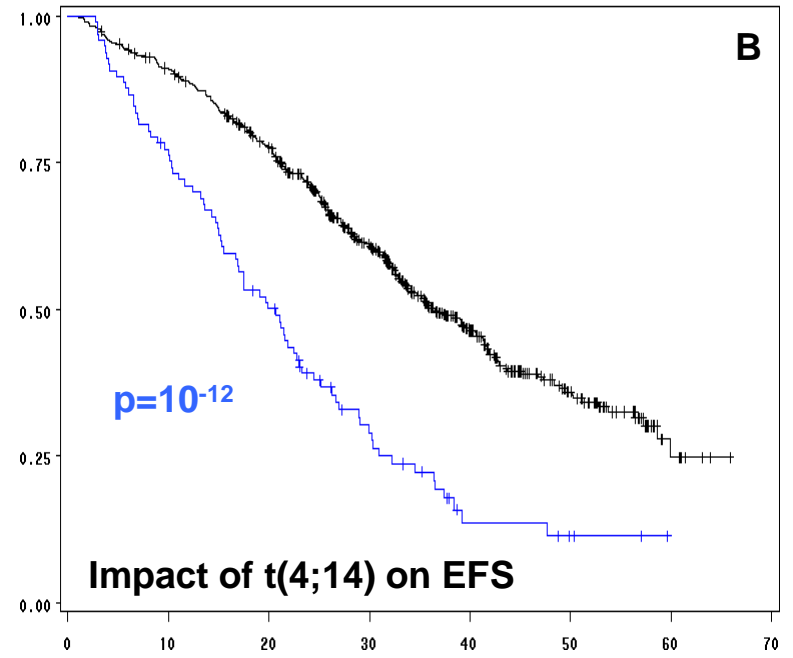
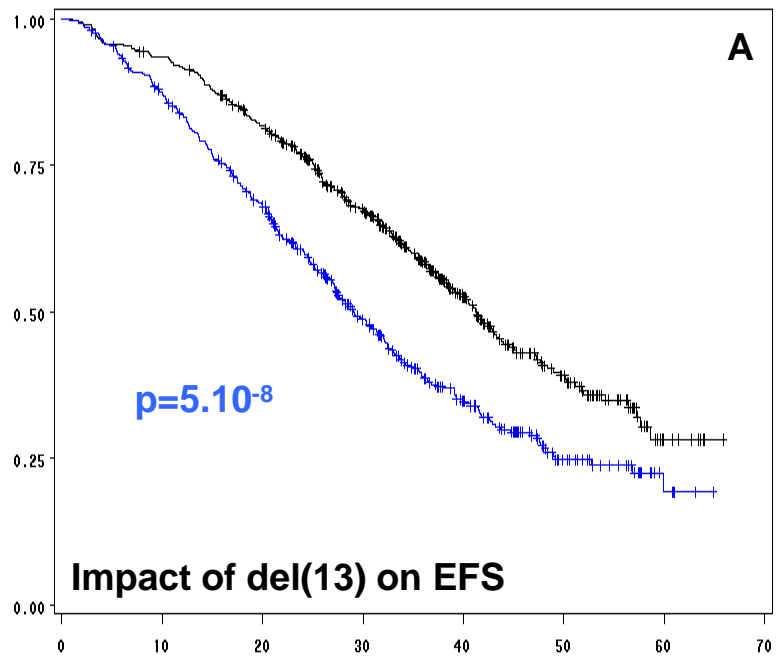


Figure 2

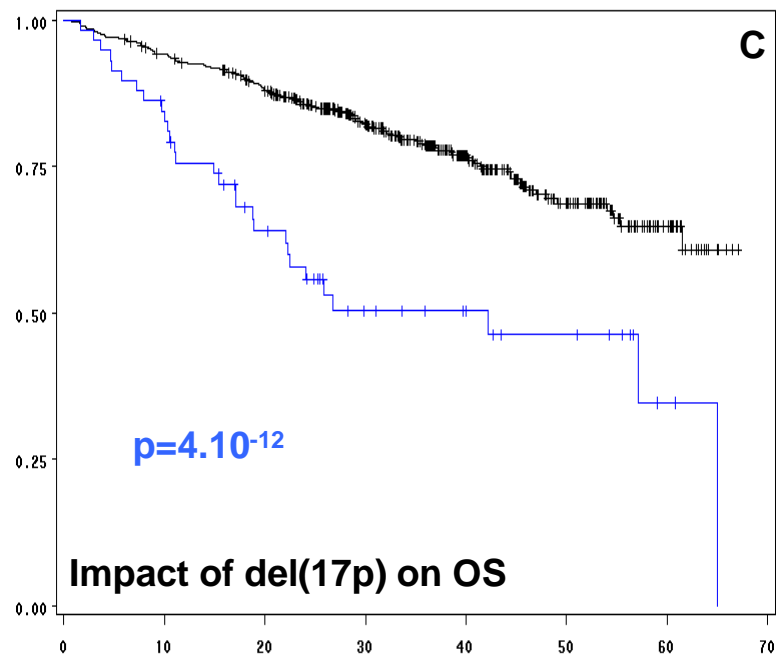
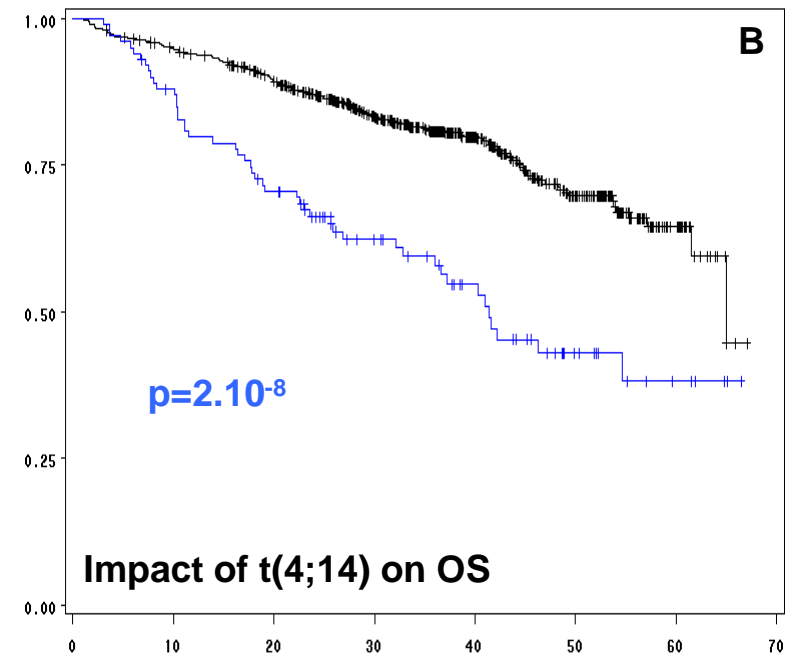
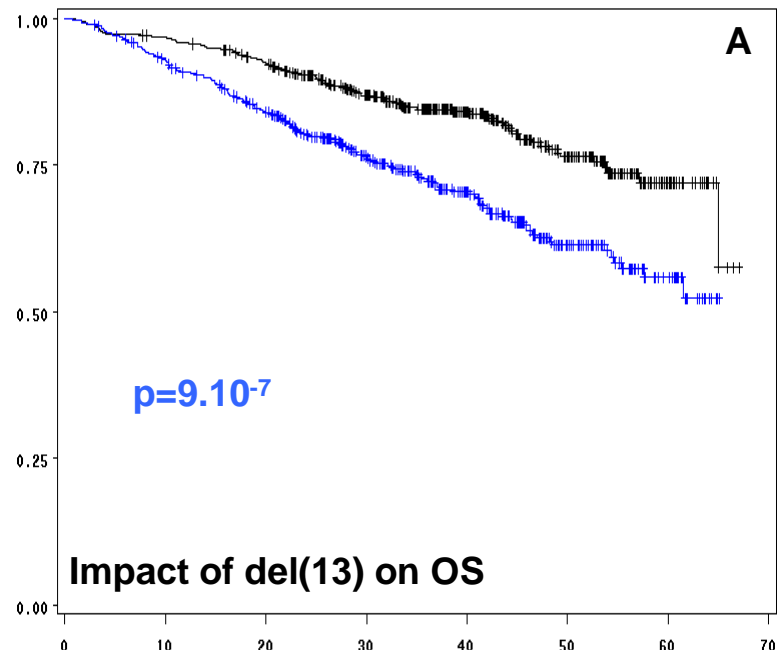


Figure 3

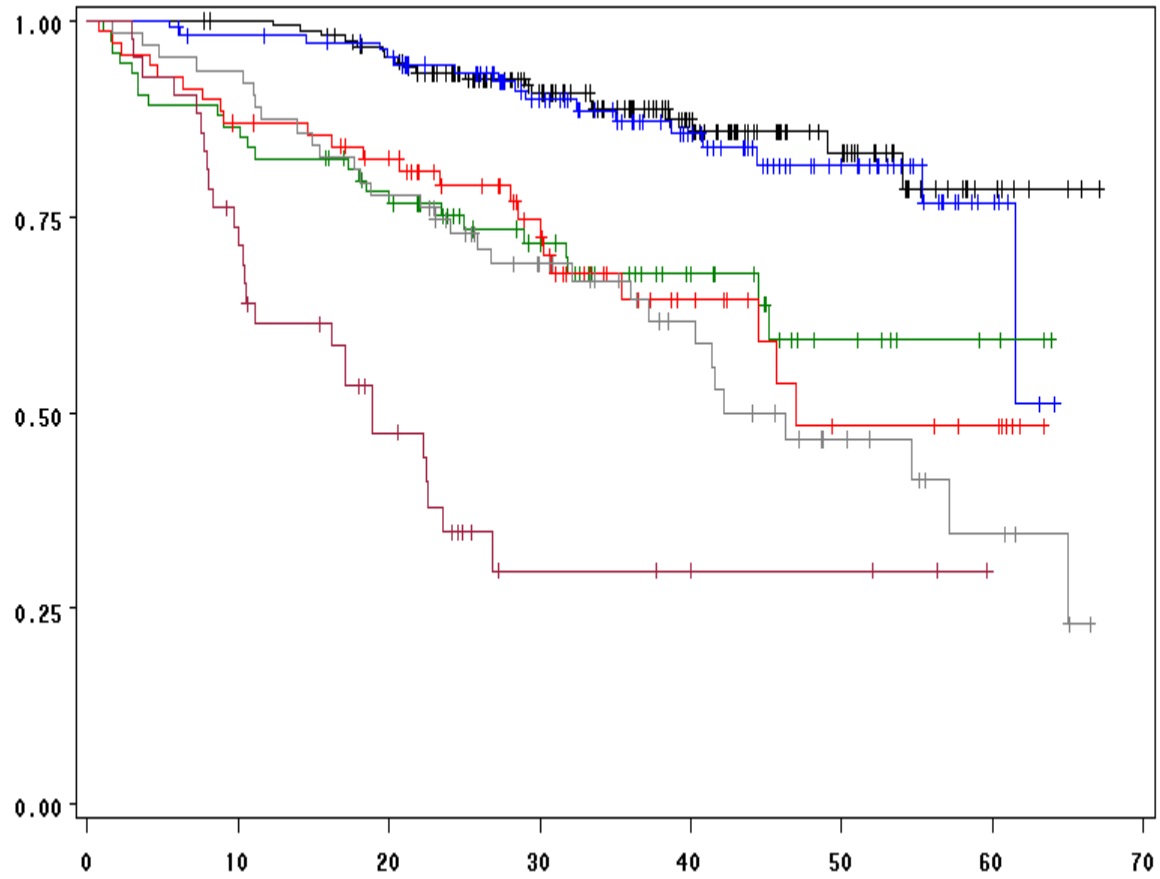


Figure 4

Impact of del(13) on event free survival (A) and overall survival (B) in patients lacking both t(4;14) and del(17p)

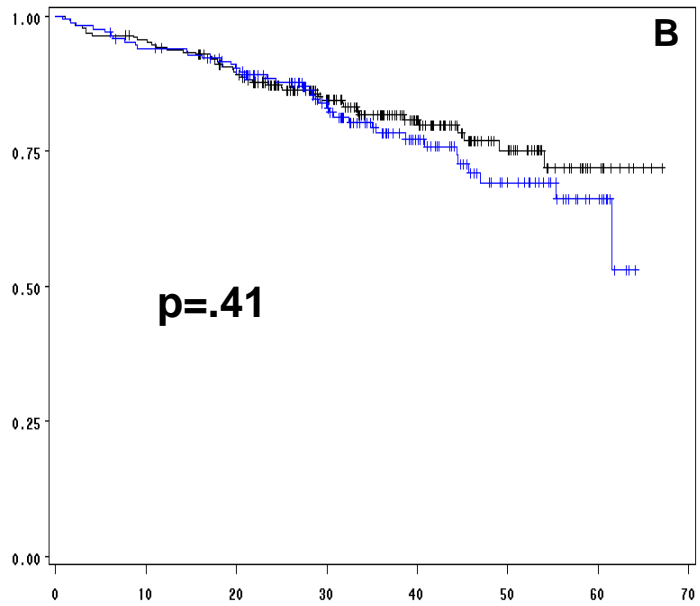
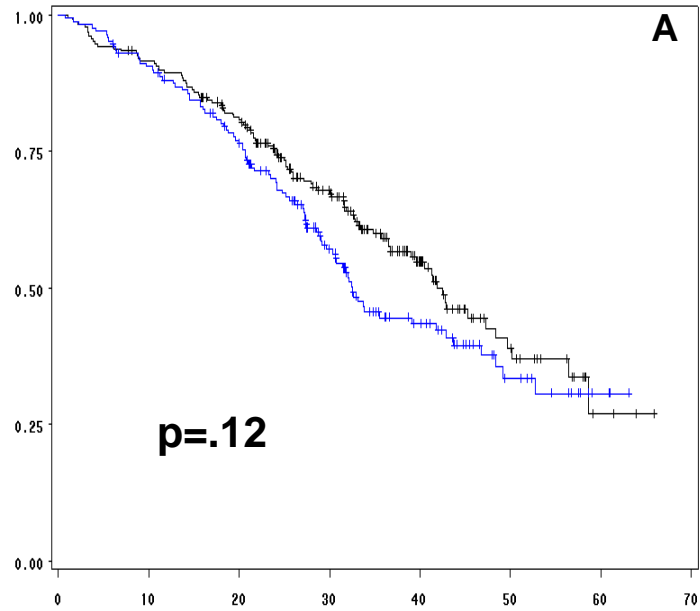
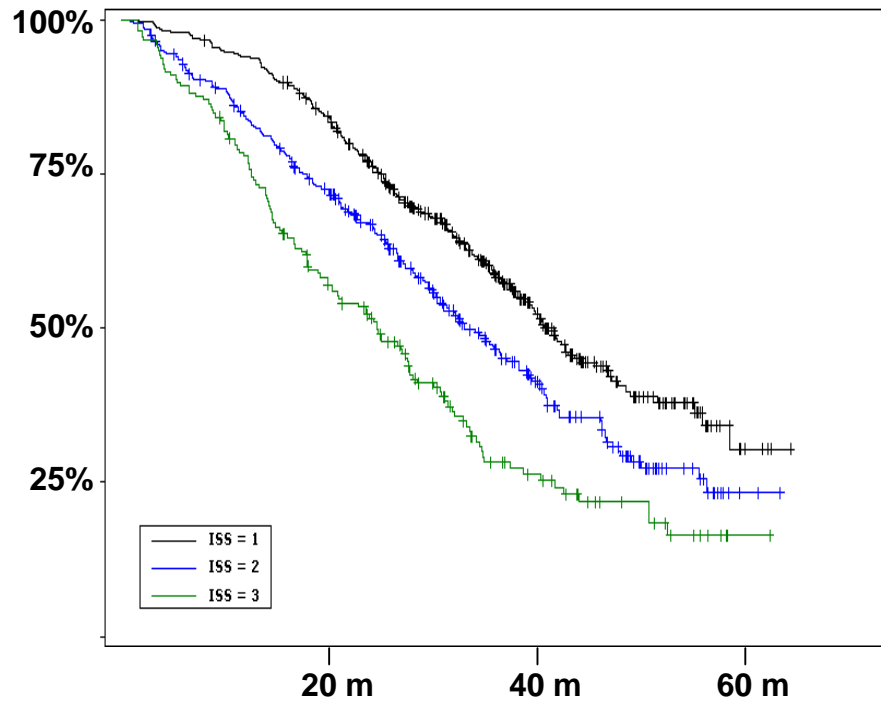
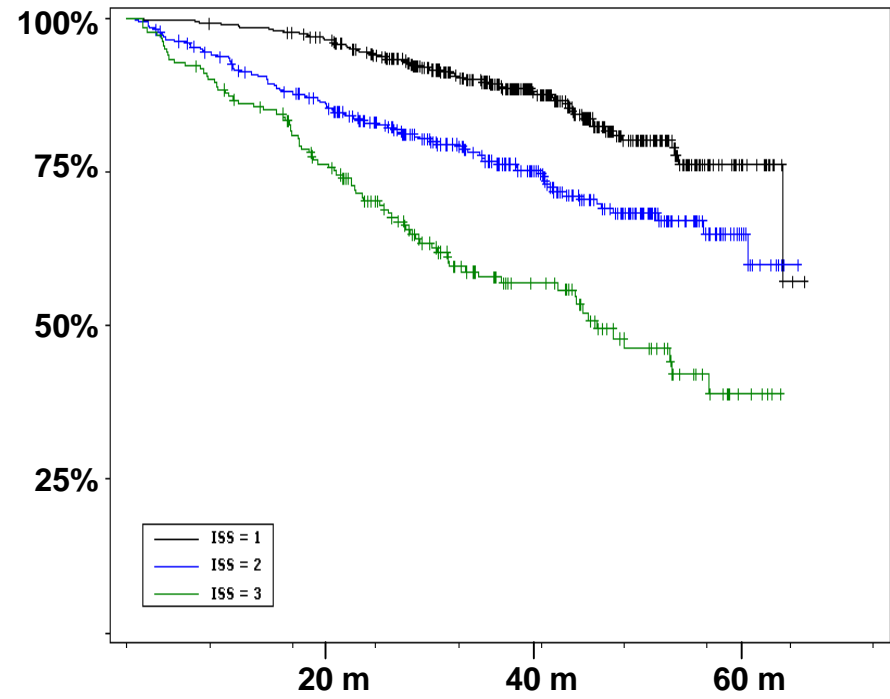


Figure 5: Survival according to the ISS stages



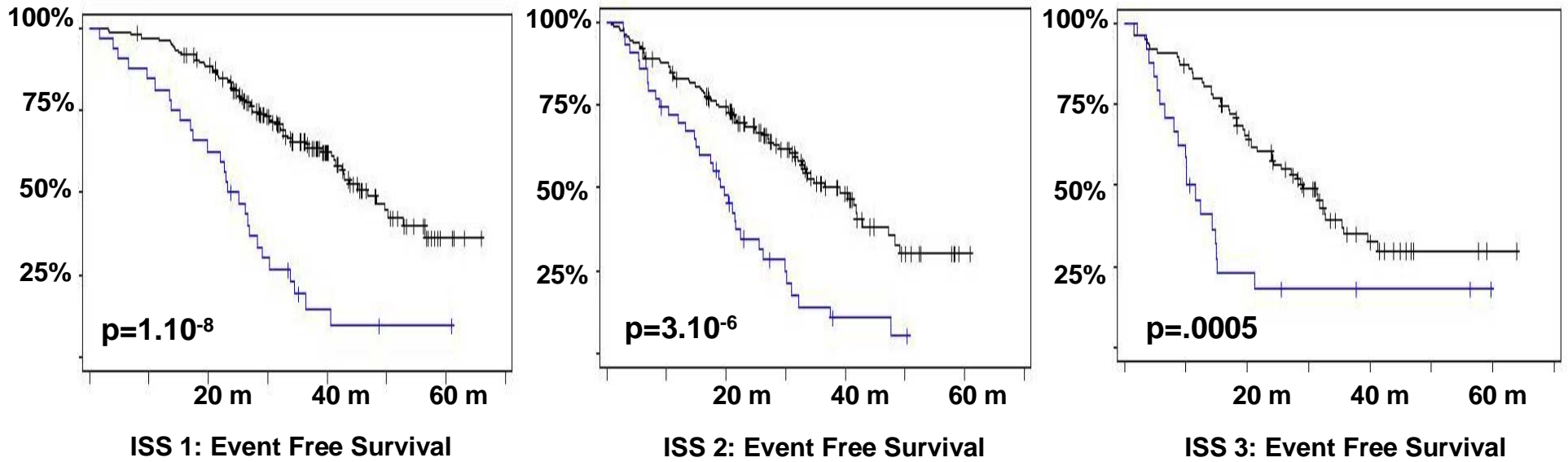
Event Free Survival



Overall Survival

Figure 6: Prognostic impact of t(4;14) and del(17p) according to ISS stages.

Panel A



Panel B

