CLINICAL AND BIOLOGIC IMPLICATIONS OF RECURRENT GENOMIC ABERRATIONS IN MYELOMA

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Non-random recurrent chromosomal abnormalities are ubiquitous in multiple myeloma (MM) and include among others translocations of the immunoglobulin (Ig) heavy chain locus (IgH). IgH translocations in MM result in the upregulation of oncogenes, and include more commonly t(11;14)(q13;q32), t(4;14)(p16;q32), and t(14;16)(q32;q23). Based on the recurrent nature of these translocations and their finding since the early stages of the plasma cell (PC) disorders we hypothesized that they would confer biologic and clinical variability. In addition, deletions of 13q14 and 17p13 have also been associated with a shortened survival. We used cytoplasmic-Ig enhanced interphase fluorescent in-situ hybridization to detect deletions (13q14 and 17p13.1), and translocations involving IgH in 351 patients treated with conventional chemotherapy entered into the Eastern Cooperative Oncology Group clinical trial E9486/9487. Translocations were frequently unbalanced with loss of one of the derivative chromosomes. The presence of t(4;14)(p16;q32) (n=42; 26 versus 45 months, P<0.001), t(14;16)(q32;q23) (n=15; 16 versus 41 months, P=0.003), -17p13 (n=37; 23 versus 44 months, P=0.005), and -13q14 (n=176; 35 versus 51 months, P=0.028) were associated with shorter survival. A stratification of patients into three distinct categories allowed for prognostication; poor prognosis group (t(4;14)(p16;q32), t(14;16)(q32;q23), and -17p13), intermediate prognosis (−13q14), and good prognosis group (all others), with median survivals of 24.7, 42.3, and 50.5 months respectively (P<0.001). This molecular cytogenetic classification identifies patients into poor, intermediate and good risk categories. More importantly it provides further compelling evidence that MM is composed of
subgroups of patients categorized according to their underlying genomic aberrations.

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Introduction

Genetic and cytogenetic abnormalities define subgroups of hematologic neoplasms, and accordingly have been associated with unique biologic, clinical and prognostic features. Recent studies with interphase fluorescent *in-situ* hybridization (FISH) indicate that all MM cells harbor chromosome abnormalities. Interphase FISH detected chromosomal abnormalities studies have also been associated with dissimilar outcomes in some reports.

We and others have proposed that specific cytogenetic abnormalities can identify groups of MM patients with unique clinical and biologic features. Abnormalities of chromosome 13 (Δ13), monosomy representing 85% of them, have an adverse prognosis in MM, when detected by metaphase analysis and interphase FISH. Likewise deletions of 17p13.1, the genomic locus of the *p53* tumor suppressor gene, have been associated with an adverse patient outcome. Translocations involving IgH (14q32) are seen in 50-60% of MM patients, and involve an array of non-random recurrent chromosomal partners, but their prognostic significance has not been tested. The three most common IgH translocations in MM are t(4;14)(p16.3;q32); t(11;14)(q13;q32), and t(14;16)(q32;q23). In this paper we evaluate and integrate the clinical and biologic relevance of the most common cytogenetic abnormalities. To do
so we used interphase FISH in a large cohort of MM patients who have had long duration of follow-up.

Patients and Methods

Patients characteristics

Patients enrolled in the Eastern Cooperative Oncology Group (ECOG) E9486 and its associated correlative laboratory clinical trial E9487 ($n = 561$) had newly diagnosed MM and have been described in detail elsewhere\textsuperscript{18}. They were randomized to receive treatment with conventional chemotherapy variations\textsuperscript{18}. The median overall survival for the whole group was 40.5 months. Patients have extensive follow-up information with the minimum follow-up of survivors being 96 months (range 96-138 months), resulting in negligible censoring. A total of 351 patients were included in this study for our analysis (Table 1) as previously described by us\textsuperscript{19}, and appeared to be no different from the larger cohort of patients when all relevant biologic and prognostic factors are considered (data not shown). Pertinent clinical and prognostic features are available for the majority of the patients including among others the PC labeling index (PCLI), $\beta_2$-microglobulin, C-reactive protein, and serum level of soluble IL-6 receptor (sIL-6R). These patients did
not have conventional karyotype analysis requested at the time of study entry and it is thus not available for comparison.

**Bone marrow samples**

IRB approval was obtained and bone marrow research samples were obtained after obtaining informed consent, and cytospin slides were stored for future use (at -70°C). Aspirate samples were enriched for mononuclear cells using the Ficol method. To improve on the specificity of the scoring process we combined interphase FISH with immune-fluorescent detection of the cytoplasmic-immunoglobulin light-chain (Fig. 1).

**Probes**

We used previously reported sets of probes to detect Δ13, t(11;14)(q31;q32), and t(4;14)(p16.3;q32)\(^{19,21,22}\). These same cohort of patients have been separately reported regarding the t(11;14)(q13;q32) and Δ13\(^{7,8}\). To detect t(14;16)(q32;q23) we used the same 14q32 chromosome probes previously described by us\(^{21}\), in combination with two BAC clones (356D21 and 484H2, Research Genetics Huntsville, AL) that localize to 16q23, and BAC clones 10205 and 10206 described by Chesi et al\(^{23}\). To test for 17p13.1, we used an LSI p53 probe from Vysis, Inc. We used standard hybridization, validation and scoring procedures as described previously by us\(^{19,21}\). We
scored 100 cells for each one of all abnormalities and recorded the percentage of cells with abnormal patterns (with special attention to the number of fusions detected for the translocations).

**Statistical analysis**

**Descriptive and Survival Analysis**

To test for association between abnormalities, or between abnormalities and other patient categorical treatment characteristics or response to treatment, Fisher’s exact test was used. To test for difference in PCLI and β2-microglobulin between patients with and without an abnormality, a Wilcoxon rank sum test was used. The distribution for overall and progression-free survival were estimated using the method of Kaplan and Meier. The log-rank test was used to test for differences in survival between groups. We decided to score 100 cells per patient to evaluate positivity and evidence of clonal heterogeneity.

**Multiple Regression Model**

A Bayesian analysis was used to evaluate the contribution of genetic abnormalities to survival. This analysis allowed us to include all the studied patients in the model, even the patients with missing data. The following clinical prognostic factors (cut-off points), dichotomized according to previously reported studies were included in the model: PCLI (<1% versus
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A Weibull distribution was used to model time to death\(^{29}\). The regression coefficients and the shape parameter, \( r \), were given ‘non-informative’ Normal and Gamma priors, respectively. Prior distributions for the covariates were assumed to be Binomial \((p, 1)\) with \( p \) distributed as Uniform \((0,1)\). The BUGS program (Bayesian inference using the Gibbs sampling algorithm)\(^{28}\) was used to estimate the coefficients and obtain 95\% credible confidence intervals. We performed an initial 500 burn-in of iterations followed by an additional 10,000 iterations. Parameter estimates are the mean and standard deviation based on the Gibbs samples; credible intervals are computed as the lower and upper \( \alpha / 2 \) percentiles from the last 10,000 iterations. The five genetic abnormalities, as well as, the clinical prognostic factors that appeared to be statistically significant (the 95\% credible interval did not contain the null value) were included in the final model.

The appropriateness of the Weibull distribution, the adequacy of the fitted multivariate model time and the validity of the Bayesian model

\( \geq 1\% \), bone marrow PC percentage \(<30\% \) versus \( \geq 30\% \), serum creatinine \(<2 \) versus \( \geq 2\text{mg/dL} \), albumin \(<3 \) versus \( >3\text{g/dL} \), hemoglobin \(<10 \) versus \( >10\text{g/dL} \), \( \beta_2\)-microglobulin \(<2.7 \) versus \( \geq 2.7\text{mg/dL} \), soluble IL-6 receptor \(<270 \) versus \( \geq 270\text{ng/mL} \), C-reactive protein \(<2 \) versus \( >2\text{mg/dL} \), serum monoclonal protein \(<1 \) versus \( >1\text{g/dL} \), and stage \((\text{I-II} \) versus \( \text{III} \).
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(“missing at random” assumption) were checked. The results obtained using the Bayesian approach were compared with the results obtained using the Cox regression model in the subset of 275 patients that had complete data.

Hierarchical Groups

Three hierarchical groups, in which one patient was allocated to one group only, were created based on hazard ratios from the results of univariate and multivariate analyses. These groups were based on whether or not patients had genetic abnormalities associated with poor, intermediate, or good prognosis.

Results

Prevalence of the abnormalities

The prevalence of chromosomal abnormalities among the 351 patients tested are as follows; t(4;14)(p16;q32) (42/332, 12.7%), t(14;16)(q32;q23) (15/323, 4.6%), t(11;14)(q13;q32) (53/336, 15.8%), deletions 17p13.1 (37/345, 10.7%), Δ13 (176/325, 54.2%). The prevalence of the abnormalities was not significantly different according to the stage of the disease or age, except for Δ13 which appeared to be more common among stage III patients \((P =0.014)\). A strong association was noted between Δ13 and the t(4;14)(p16.3;q32) as we have previously reported (38/42 patients)\((P < 0.001)^{22}\), but this association
was not present in patients with t(14;16)(q32;q23) (8/13 patients) or
t(11;14)(q13;q32) (24/51 patients). A significant positive correlation between
deletion 17p13.1 and t(14;16)(q32;q23) was observed (5/10 patients 33%) ($P = 0.018$), and was suggested for t(4;14)(p16;q32) (8/42, 20%). In contrast
17p13.1 deletions had a lower incidence in patients with t(11;14)(q13;q32)($P = 0.027$).

**Translocation patterns and relations**

In this cohort of patients all translocations were mutually exclusive; that
is there were no patient with two coexistent translocations. However, many
patients had combinations of a translocation and deletion(s) of 17p13.1 and/or
$\Delta$13. The median percent of cells with an abnormality was greater than 80% for all abnormalities. IgH translocations were usually seen in greater than 95% of cells. Deletions 17p13.1 were seen in less than 50% of cells in 11 of 37 cases (30%). Using the specific sets of probes we found that of 348 evaluable
patients, 139 (40%) had one chromosomal abnormalities detected, 77 (22%)
had two, and 10 (3%) had three abnormalities. Using these probes a total of
122 patients had no abnormalities detected.
Prognostic features including the PCLI and \( \beta_2 \)-microglobulin

Patients had unique biologic and prognostic features according to their baseline prognostic features (Table 1). The PCLI was significantly higher among patients with \( \Delta 13 \) \( (P = 0.03) \), \( t(14;16)(q32;q23) \) \( (P = 0.02) \), or deletion 17p13.1 \( (P = 0.01) \). Serum levels of \( \beta_2 \)-microglobulin appeared to be significantly higher in patients with deletion 17p13.1 \( (P = 0.03) \). Deletions of 17p13.1 were significantly associated with hypercalcemia \( (P = 0.009) \) and soft tissue plasmacytomas \( (P = 0.0053) \). The use of lambda light chain was favored in patients with \( t(14;16)(q23;q32) \) \( (P = 0.05) \). Serum levels of sIL6-R were higher in patients with \( \Delta 13 \) \( (P = 0.003) \), \( t(4;14)(p16.3;q32) \) \( (p=0.025) \), \( t(14;16)(q32;q23) \) \( (P = 0.009) \), and deletions of 17p13.1 \( (P = 0.006) \). Patients with \( t(4;14)(p16.3;q32) \) were significantly more likely to have a serum monoclonal protein greater than 3g/dL \( (P = 0.019) \). Light-chain only disease was slightly more common among those patients with \( t(11;14)(q13;q32) \) \( (28\%) \) among patients with \( t(11;14)(q13;q32) \) and 16% in those without \( t(11;14)(q13;q32) \), Fisher’s exact \( P \) value=0.04). The IgA isotype was slightly more common among patients with the \( t(4;14)(p16.3;q32) \) but was not significant \( (P >0.2) \). No trend was observed among patients with \( t(14;16)(q32;q23) \).
Balanced versus unbalanced translocations

A predominant pattern of one fusion signal only was seen in 56 of 103 (51%) patients with evidence of a translocation by the fusion strategy. When one only considers cases where the predominant pattern was seen in greater than 90% of the clonal cells the total was 34 patients (33% of all IgH translocations). This is remarkably different to what has been previously believed in MM as balanced translocations have been assumed. (Table 2) A recent publication by Keats and colleagues demonstrates that using RT-PCR based strategies they find that up to one third of patients with the t(4;14)(p16.3;q32) have unbalanced IgH translocations30.

Response to treatment

Among patients evaluable for response, those with Δ13 had a lower likelihood of an objective response than those without the abnormality (Table 3). Otherwise there were no major differences noted.

Survival analysis

On the univariate analysis, patients with t(4;14)(p16.3;q32), t(14;16)(q32;q32), deletions of 17p13.1 and Δ13 had a significantly worse overall survival (Fig. 2 and Table 3). Progression free survival was significantly worse in patients with t(4;14)(p16.3;q32), t(14;16)(q32;q32), and
Δ13, and was of marginal significance in patients with deletions of 17p13.1 (Table 3).

**Multiple Regression Model**

When adjusting for the clinical factors that were statistically significant, as well as for other genetic abnormalities, t(4;14)(p16.3;q32) and t(14;16)(q32;q23) had the highest hazard ratios (1.78 and 1.67, respectively), t(4;14)(p16.3;q32) being statistically significant and t(14;16)(q32;q23) marginally significant. Deletion of 17p13.1 was marginally significant with an intermediate hazard ratio (1.34) with respect to the other four genetic abnormalities (Table 4). Δ13 were also statistically significant, with a hazard ratio equal to 1.28. These results were similar to those obtained using the Cox regression model for only the 275 cases with complete data. The hazard ratios (95% CI) of the genetic abnormalities in the Cox regression model, which also included creatinine, PCLI and bone marrow PC percentage as covariates, were 1.69 (1.15, 2.49) for t(4;14)(p16.3;q32), 1.42 (0.75, 2.66) for t(14;16)(q32;q23), 1.47 (0.97, 2.20) for -17p13.1, 1.35 (1.04, 1.74) for Δ13, and 0.94 (0.66, 1.34) for t(11;14)(q13;q32).
Prognostic Groups

Three hierarchical groups identified three distinct prognostic groups (log-rank $P$ value<0.001). The groups are as follows: Poor prognosis group- patients with t(4;14)(p16.3;q32) and/or t(14;16)(q32;q23) and/or deletion 17p13.1 ($n = 66$); intermediate prognosis- patients with Δ13, but none of t(4;14)(p16.3;q32), t(14;16)(q32;q23), or deletion 17p13.1 ($n = 103$); and good prognosis- patients with t(11;14)(q13;q32) only or none of the abnormalities tested ($n = 106$). Their median survival times were 24.7 months, 42.3 months and 50.5 months respectively (Fig. 3).

Discussion

Summary

In this study we find subgroups of MM patients classified according to their underlying cytogenetic abnormalities, and show that these abnormalities alone can establish prognostic categories. Our study provides conclusive clinical evidence that MM is not a single uniform disorder, but rather a group of them that can be defined by their underlying cytogenetic anomalies supported by this biologic variability\textsuperscript{11}. Patients with the t(4;14)(p16.3;q32), t(14;16)(q32;q23), and deletion of 17p13.1 have a significantly worse prognosis than others. We suspect this observation is likely due to the
upregulation of specific oncogenes involved in these translocations and to loss of the tumor suppressor gene \(p53\) respectively. We previously demonstrated that chromosomal abnormalities define unique presenting factors for MM and may be associated with specific features such as the oligosecretory variant, \(\lambda\)-light chain usage or ploidy status\(^7,8\).

**Biology of IgH translocations in general**

Between 50\% to 60\% of MM patients harbor IgH translocations\(^31-33\), and these translocations have been detected since the very early stages of the PC neoplasms (i.e. MGUS)\(^34,35\). This is consistent with IgH translocations being primary events, as is seen in the mouse plasmacytoma model where they result in \(c\)-\(myc\) upregulation\(^36\). Of interest, all IgH translocations in MM appear to be upregulating proliferation genes\(^12\). We conclude that while translocations may be an early and important step\(^34,35\), they are not sufficient in humans for malignant transformation and more likely result in the initial clone-immortalizing event. It is important to note that the translocations that impart a poor prognosis in the active MM stage have no known effect on prognosis when they are detected in MGUS. In fact we have found that patients with MGUS and the t(4;14)(p16.3;q32) or t(14;16)(q32;q23) may remain without progression to MM for prolonged periods of time\(^35\).
Genomic convergence and translocations

Despite ongoing genomic instability, IgH translocations are highly conserved, as they are not lost with advancement through the different stages of the PC neoplasms, and in fact are clonally selected as they are seen in the majority of the clonal cells\textsuperscript{10,37}. Roschke and colleagues, exploring genomic instability in human colorectal and ovarian cancer cell lines, have previously explored the model of “signature karyotypes”, and our working hypothesis is consistent with it\textsuperscript{38}. Here we show that in virtually all abnormal MM cases IgH translocations involve a large percentage of the PCs. We observe similar patterns with Δ13 but not with –17p13. This finding is in great contrast with aneuploid clones that more commonly only affect a fraction of cells\textsuperscript{39,40}. While the situation is less clear in MGUS, it is also suggested that in many cases translocations will involve the majority of the clonal cells\textsuperscript{34,35}.

\textit{t(4;14)(p16.3;q32)}

We have first reported the negative prognostic implications of both the t(4;14)(p16.3;q32) and the t(14;16)(q32;q23) in MM patients treated with chemotherapy\textsuperscript{41}. Similar prognostic effects have been shown by Moreau and colleagues for patients with t(4;14)(p16.3;q32) treated with high dose chemotherapy\textsuperscript{9}. The mechanisms resulting in the negative prognostic associations with the t(4;14)(p16.3;q32) are not known.
In contrast to other B-cell malignancies IgH translocations in MM can deregulate two or more oncogenes\textsuperscript{12}. This is because in MM IgH translocations occur into switch regions causing segregation of the E\textsubscript{μ} and 3’\textgreek{α} enhancers. An example of this is the t(4;14)(p16.3;q32). While other genes may be upregulated by the t(4;14)(p16.3;q32)\textsuperscript{42}, FRGR3 and MMSET are best characterized. However, not in all cases of a t(4;14)(p16.3;q32) is FGFR3 upregulated, and it has been found that der14 chromosome can be lost in primary samples or cell lines (\textit{Michael Kuehl, personal communication})\textsuperscript{30}. This implies that MMSET deregulation is needed for clone survival. The probes used in this study bracket all reported human MM breakpoints (for all translocations) and should in theory always result in two detectable fusion signals. However, in this study we have found that IgH translocations in MM will frequently be unbalanced (\textbf{Table 2}).

\textit{t(14;16)(q32;q23)}

Unlike the study by Avet-Loiseau, we have found the t(14;16)(q23;q32) as recurrent in MM\textsuperscript{10}. We observed the t(14;16)(q32;q23) in 5\% of patients, and had a clear association with an adverse outcome, with a shorter survival and features of aggressiveness. We have also detected the t(14;16)(q32;q23) in MGUS without transformation to MM\textsuperscript{35}, but nevertheless when the abnormality is seen in the active stages of the disease still confers an
aggressive phenotype. While c-maf upregulation is believed to be culprit, a recently described gene, WWOX, is also disrupted by breakpoints at 16q23\(^43\). A possible tumor suppressor role is being sought for this gene, that spans several hundred kilobases at 16q32, and is located at the fragile site Fra16D\(^43\). C-maf, has been shown to have transforming activity in chicken fibroblasts\(^44\).

The t(14;16)(q32;q23) is observed in 25% of human MM cell lines but is only seen in 5% of primary MM samples. In one of these human MM cell lines, KMS-11, the 16q23 breakpoint places c-maf at greater than 700 kb of the IgH enhancer in the translocated allele, without evidence of intervening deletion or inversion (\textit{R Fonseca, unpublished observations}). This highlights the possibility that other oncogenes than those described to date may be upregulated by any IgH translocation, other than those current believed important for pathogenesis.

**Chromosome 13 abnormalities**

Our study confirms that Δ13 have a negative impact on prognosis. Others and we have found that Δ13 detected by FISH is an independent prognostic variable on a multivariate analysis\(^4,6\). The genes associated with the negative prognostic implications of Δ13 have yet to be defined. Detailed molecular analysis has revealed that in the majority of cases Δ13 are indicative of monosomy\(^19,45\). While a minimally deleted region has been postulated as
being in 13q14 other sites may be involved as well\textsuperscript{19,45}. The role of Δ13 in the
pathogenesis of MM is still being elucidated. In the setting of widespread
genomic instability, chromosome 13 is almost never seen as trisomic
suggesting clonal intolerance to the gain\textsuperscript{46}.

**Deletion of 17p13.1 (p53)**

Deletions at the p53 locus also confer an adverse prognosis, even when
they are only observed in a small proportion of patients\textsuperscript{15}. While most other
abnormalities (*i.e.* IgH translocations and Δ13) showed little heterogeneity,
p53 deletions could be seen in smaller percents of cells and suggest early
clonal evolution. Furthermore, patients with this specific abnormality were
more likely to have other features of aggressiveness such as plasmacytomas
and hypercalcemia. All this information suggest that even when p53 deletions
may be detected at the time of diagnosis, it is likely a marker of an advanced
clone.

**Therapeutic implications**

As targeted therapy evolves, different treatment interventions will have
variable success, depending on the underlying genetic nature of the clone\textsuperscript{47}.
For instance, the development of effective *MMSET* or *FGFR3* small molecule
inhibitors may allow for patients with t(4;14)(p16.3;q32) to become a better
prognostic category. The use of inhibitors of the cyclin D1/CDK pathways, such as flavopiridol, seems especially suited for patients with t(11;14)(q13;q32). It is also worth noting that it appears that specific treatments may be better tailored for patients with specific chromosomal abnormalities. A comparison of our results (in patients treated with conventional chemotherapy) to those of Moreau (in patients treated with high dose therapy) suggests that high-dose chemotherapy provides little, or no, survival advantage for patients with Δ13 or t(4;14)(p16.3;q32)\textsuperscript{8,9}. In contrast it appears that high-dose chemotherapy provides a significant survival increment for patients with t(11;14)(q13;q32)\textsuperscript{7,9}. While these observations are speculative for now as they are based on a retrospective comparison, they are highly provocative and in need of confirmation in prospective clinical trials.

**Statistical aspects**

A Bayesian approach was used in this study to assess the impact of genetic abnormalities on survival adjusting for known clinical prognostic factors. The advantage of the Bayesian analysis is that it allows including all the subjects in the model, even those that have missing data in their covariates. In our study sample 22% of subjects had missing data, either in the genetic abnormalities or in the clinical variables. Imputation of missing data is done in the Gibbs sampling framework by treating missing values as additional
unknown quantities and randomly selecting values from their conditional distributions. Conditional distributions are a function of the observed individual data and the current sampled values of the other missing data for a particular individual. There was no indication of non-random “missingness” in our data, one of the assumptions of the Bayesian analysis. Also, the assumptions of Weibull distribution, proportional hazards and adequacy of the multivariate model were assessed with satisfactory results. When the results of the Bayesian approach (which included all the studied 351 patients) were compared with those of the Cox proportional hazards regression (which included only the 275 patients with complete data), the hazard ratios were similar. Differences were observed mostly for the genetic abnormalities with the smallest prevalence: (t(14;16)(q32;q23) and -17p13).

It is important to cautiously interpret the hierarchical group survival analysis, being that patients in the poor prognosis group could have more abnormalities than patients in the intermediate or good prognosis groups. For instance, patients in the poor prognosis group could possibly have Δ13 deletion in addition to one or more of the poor prognosis abnormalities. To make sure this was not the only reason that patients in the poor prognosis group did poorly, we switched the order of the hierarchical grouping. In the new grouping patients with Δ13 were in one group, patients with any of the three
(t(4;14)(p16.3;q32), t(14;16)(q32;q23), deletion 17p13.1) poor prognosis abnormalities, but not Δ13 were in another group, and patients with only t(11;14)(q13;q32) or none of the five tested abnormalities were in the third group. While the median survival times differed slightly from the originally hierarchical grouping, the trend in median survival times was the same.

Patients with the poor prognosis abnormalities (t(4;14)(p16.3;q32), t(14;16)(q32;q23), deletion 17p13.1) did worse than patients with Δ13, and those patients both did worse than patients with only t(11;14)(q13;q32) or none of the five tested abnormalities. We thus conclude that patients with t(4;14)(p16.3;q32), t(14;16)(q32;q23), or deletion 17p13.1 seem to make up a poor prognosis group.
Figure Legends

Figure 1: PCs with both the normal (A) and abnormal pattern of hybridization (B) to test for Δ13.

The depicted PCs show a normal and abnormal pattern of hybridization. In all panels we show the blue fluorescence of the cytoplasm allowing the clone specific interphase FISH scoring. Panel A shows a cell with the normal configuration of two pairs of signals for the probes localizing to the centromere 17 (green) and the 17p13.1 (LSI p53) probe (red). The cell in panel B shows a cell with deletion of 17p13.1. There are two green signals arising form the centromeric probe but only one red signal from the p53 locus probe. Panel C shows a normal configuration of probes used to detect the t(14;16)(q32;q23). The locus specific 14q32 probes are labeled in green and the 16q23 probes are labeled in red. In panel D we show a cell with fusion of probes for 14q32 (green) and 16q23 (red). The two signals in proximity generate a fusion. If a significant number of cells scored showed this pattern a patients is said to have a translocation.

Figure 2: Overall survival of patients stratified by the presence or absence of each one of the specific cytogenetic abnormalities showing statistical significance.
The significance values are expressed next to each curve and correspond to the log-rank test. The x-axis values represent time since diagnosis and is expressed in months. The results for the t(11;14)(q13;q32) are not shown as it was not statistically significant.

Figure 3: Overall survival of patients stratified by the hierarchical classification model proposed by our study.

The survival curves show clear separation of patients into the good, intermediate and poor prognosis category, a difference that was statistically significant. Groups were formed by the stratification according to the presence or absence of specific genetic abnormalities. The poor prognosis group includes patients with −17p13.1, t(4;14)(p13;q32), and/or t(14;16)(q32;q23); the intermediate prognosis group includes those patients with Δ13 that did not have the aforementioned abnormalities; and the better prognosis group includes those remaining patients including those with the t(11;14)(q13;q32) and none of the aforementioned abnormalities.
Table 1

**Baseline clinical and laboratory descriptive features of patients by abnormality. Numbers in brackets denote range.**

<table>
<thead>
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<th>Variable</th>
<th>All (n = 351)</th>
<th>t(4;14)(p16;q32) (n = 42)</th>
<th>t(14;16)(q32;q23) (n = 15)</th>
<th>t(11;14)(q13;q32) (n = 53)</th>
<th>Δ13 (n = 176)</th>
<th>Del 17p13.1 (n = 37)</th>
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<td>91</td>
<td>88</td>
<td>84</td>
</tr>
<tr>
<td>2-4 (%)</td>
<td>14</td>
<td>10</td>
<td>0</td>
<td>9</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td><strong>Plasmacytoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>6</td>
<td>11</td>
<td>24</td>
</tr>
<tr>
<td><strong>Lytic bone lesions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (%)</td>
<td>61</td>
<td>62</td>
<td>54</td>
<td>62</td>
<td>60</td>
<td>70</td>
</tr>
<tr>
<td><strong>Hypercalcemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca²⁺ &gt; 12mg/dL (%)</td>
<td>24</td>
<td>21</td>
<td>27</td>
<td>23</td>
<td>26</td>
<td>43</td>
</tr>
<tr>
<td><strong>Serum M component</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present (≥1g/dL) (%)</td>
<td>83</td>
<td>90</td>
<td>73</td>
<td>72</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Absent (%)</td>
<td>17</td>
<td>10</td>
<td>27</td>
<td>28</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td><strong>Urine M component</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present (detectable) (%)</td>
<td>72</td>
<td>69</td>
<td>67</td>
<td>68</td>
<td>76</td>
<td>84</td>
</tr>
<tr>
<td>Absent (%)</td>
<td>25</td>
<td>24</td>
<td>33</td>
<td>30</td>
<td>20</td>
<td>14</td>
</tr>
<tr>
<td>Unknown (%)</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

From www.bloodjournal.org by guest on October 23, 2017, For personal use only.
<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 351)</th>
<th>t(4;14)(p16;q32) (n = 42)</th>
<th>t(14;16)(q32;q23) (n = 15)</th>
<th>t(11;14)(q13;q32) (n = 53)</th>
<th>Δ13 (n = 176)</th>
<th>Del 17p13.1 (n = 37)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Light chain type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>κ (%)</td>
<td>63</td>
<td>48</td>
<td>40</td>
<td>60</td>
<td>59</td>
<td>54</td>
</tr>
<tr>
<td>λ (%)</td>
<td>33</td>
<td>50</td>
<td>60</td>
<td>34</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td>Unknown (%)</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral blood PCs (%)</td>
<td>0% [0.93]</td>
<td>0% [0.8]</td>
<td>0% [0.12]</td>
<td>0% [0.10]</td>
<td>0% [0.93]</td>
<td>0% [0.56]</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.2 [0.4,4.9]</td>
<td>1.2 [0.6,4.8]</td>
<td>1.1 [0.4,4.9]</td>
<td>1.3 [0.5,4.7]</td>
<td>1.2 [0.5,4.8]</td>
<td>1.2 [0.4,4.8]</td>
</tr>
<tr>
<td>β2-microglobulin (mg/dL)</td>
<td>3.7 [0.6,64.0]</td>
<td>3.9 [0.6,21.3]</td>
<td>5.4 [1.0,64.0]</td>
<td>4.0 [0.9,18.4]</td>
<td>3.8 [0.6,30.3]</td>
<td>4.2 [1.0,23.9]</td>
</tr>
<tr>
<td>PCLI (% of PC)</td>
<td>0.4 [0.15.4]</td>
<td>0.6 [0.13.2]</td>
<td>1.0 [0.10]</td>
<td>0.4 [0.5,9]</td>
<td>0.6 [0.13.2]</td>
<td>1.1 [0.10.9]</td>
</tr>
</tbody>
</table>
Table 2

**Prevalence of unbalanced translocations**

<table>
<thead>
<tr>
<th>Total patients with each translocation</th>
<th>Patients with predominantly one signal</th>
<th>All patients with each translocation</th>
<th>Patients with one signal %</th>
<th>Percent of cells with only one fusion &gt; 90%</th>
<th>Percent of cells with only one fusion &gt; 75% but less than 90%</th>
<th>Percent of cells with only one fusion &lt; 75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)(p16.3;q32)</td>
<td>14</td>
<td>35</td>
<td>40%</td>
<td>3/14</td>
<td>21%</td>
<td>6/14</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>35</td>
<td>53</td>
<td>66%</td>
<td>25/35</td>
<td>71%</td>
<td>4/35</td>
</tr>
<tr>
<td>t(14;16)(q32;q23)</td>
<td>7</td>
<td>15</td>
<td>47%</td>
<td>6/7</td>
<td>86%</td>
<td>1/7</td>
</tr>
</tbody>
</table>

|                                | 56                                    | 34                                | 11                          | 11                                      |                                                 |                                         |                                          |                                          |                                          |
Table 3

**Overall Survival (OS), progression-free survival (PFS) and objective response (OR) to treatment by abnormality**

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>N</th>
<th>Median OS with abnormality in months (95% CI)</th>
<th>Median OS without abnormality in months (95% CI)</th>
<th>P value</th>
<th>Median PFS with abnormality in months (95% CI)</th>
<th>Median PFS without abnormality in months (95% CI)</th>
<th>p value</th>
<th>OR* with abnormality n (%)</th>
<th>OR* without abnormality n (%)</th>
<th>p value</th>
<th>5 yr. OS rate with abnormality (%)</th>
<th>5 yr. OS rate without abnormality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14)(p16;q32)</td>
<td>332</td>
<td>26 (21,33)</td>
<td>45 (39,50)</td>
<td>&lt;.001</td>
<td>17 (13,21)</td>
<td>31 (28,34)</td>
<td>&lt;.001</td>
<td>26 (62)</td>
<td>197 (69)</td>
<td>.38</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>t(14;16)(q32;q23)</td>
<td>323</td>
<td>16 (13,22)</td>
<td>41 (37,48)</td>
<td>.003</td>
<td>9 (6,13)</td>
<td>30 (27,32)</td>
<td>.003</td>
<td>8 (53)</td>
<td>204 (68)</td>
<td>.27</td>
<td>13</td>
<td>29</td>
</tr>
<tr>
<td>t(11;14)(q13;q32)</td>
<td>336</td>
<td>50 (37,60)</td>
<td>39 (36,44)</td>
<td>.332</td>
<td>33 (28,45)</td>
<td>27 (25,31)</td>
<td>.590</td>
<td>39 (78)</td>
<td>187 (67)</td>
<td>.14</td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td>Deletion 17p13</td>
<td>345</td>
<td>23 (20,36)</td>
<td>44 (39,49)</td>
<td>.005</td>
<td>17 (11,24)</td>
<td>30 (27,33)</td>
<td>.051</td>
<td>25 (68)</td>
<td>208 (69)</td>
<td>.85</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Δ13</td>
<td>325</td>
<td>35 (29,41)</td>
<td>51 (41,57)</td>
<td>.028</td>
<td>25 (21,29)</td>
<td>33 (30,37)</td>
<td>.030</td>
<td>109 (63)</td>
<td>108 (74)</td>
<td>.04</td>
<td>22</td>
<td>38</td>
</tr>
</tbody>
</table>

*: The number of patients evaluable for response may be slightly smaller than N given above.
### Table 4

**Multivariate Bayesian analysis for survival using the Weibull proportional hazards model. (n=351)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>-10.760</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(4;14)(p16;q32) <em>(present vs absent)</em></td>
<td>0.574</td>
<td>0.179</td>
<td>1.78 (1.23, 2.50)</td>
</tr>
<tr>
<td>t(14;16)(q32;q23) &quot; &quot;</td>
<td>0.513</td>
<td>0.286</td>
<td>1.67 (0.92, 2.83)</td>
</tr>
<tr>
<td>Deletion 17p13 &quot; &quot;</td>
<td>0.291</td>
<td>0.188</td>
<td>1.34 (0.92, 1.93)</td>
</tr>
<tr>
<td>Δ13 &quot; &quot;</td>
<td>0.244</td>
<td>0.119</td>
<td>1.28 (1.01, 1.61)</td>
</tr>
<tr>
<td>t(11;14)(q13;q32) &quot; &quot;</td>
<td>-0.213</td>
<td>0.163</td>
<td>0.81 (0.58, 1.11)</td>
</tr>
<tr>
<td>Creatinine (≥ 2 vs &lt; 2 mg/dL)</td>
<td>0.580</td>
<td>0.165</td>
<td>1.79 (1.29, 2.44)</td>
</tr>
<tr>
<td>PC labeling index (≥ 1 vs &lt; 1 %)</td>
<td>0.441</td>
<td>0.123</td>
<td>1.55 (1.22, 1.97)</td>
</tr>
<tr>
<td>C-reactive protein (≥2 versus &lt; 2 mg/dL)</td>
<td>0.435</td>
<td>0.175</td>
<td>1.54 (1.09, 2.16)</td>
</tr>
<tr>
<td>Bone marrow involvement (≥ 30% versus &lt; 30%)</td>
<td>0.354</td>
<td>0.116</td>
<td>1.43 (1.14, 1.79)</td>
</tr>
</tbody>
</table>
References

especially in patients receiving intensive chemotherapy. Blood. 2002;100:1579-1583


13. Tricot G, Barlogie B, Jagannath S, Bracy D, Mattox S, Vesole DH, Naucke S, Sawyer JR. Poor prognosis in multiple myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. Blood. 1995;86:4250-4256


in multiple myeloma identified by interphase FISH usually denote large deletions of the q-arm or monosomy. Leukemia. 2001;15:981-986


22. Fonseca R, Oken M, Greipp P. The t(4;14)(p16.3;q32) is Strongly Associated with Chromosome 13 Abnormalities (Δ13) in Both Multiple Myeloma (MM) and MGUS. Blood. 2001;98:1271-1272


Bataille R. High incidence of translocations t(11;14)(q13;q32) and t(4;14)(p16;q32). Cancer Res. 1998;58:5640-5645


42. Still IH, Vince P, Cowell JK. The third member of the transforming acidic coiled coil-containing gene family, TACC3, maps in 4p16, close to translocation breakpoints in multiple myeloma, and is upregulated in various cancer cell lines. Genomics. 1999;58:165-170.


Genomic aberrations in myeloma

**Fig 1**

Panel A: CEP17 and LSI p53

Panel B: CEP17 and LSI p53

Panel C: 14q32 and 16q23

Panel D: Fusion indicating translocation

14q32 and 16q23
Genomic aberrations in myeloma

Fonseca et al.

\[ t(4;14)(p16.3;-17p13.1) \]

\[ P=0.005 \]

\[ t(14;16)(q32;13) \]

\[ P<0.001 \]

\[ t(4;14)(p16.3;13) \]

\[ P=0.028 \]

\[ t(14;16)(q32;q23) \]

\[ P=0.002 \]

\[ -17p13.1 \]

\[ P=0.005 \]

Fig 2

Survival probability

\[ \Delta13 \]
Genomic aberrations in myeloma

Fonseca et al.

Fig 3

Survival probability

0.0
0.2
0.4
0.6
0.8
1.0

P<0.001

All others including t(11;14)

Δ13

t(4;14) or
t(14;16) or

-17p13

0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150

Months

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