Trisomies in multiple myeloma: impact on survival in patients with high-risk cytogenetics

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**Short Title:** Trisomies and outcome in myeloma

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ABSTRACT

Routine incorporation of fluorescence in situ hybridization (FISH) testing into the multiple myeloma work-up has lead to better appreciation of the heterogeneity of genetic abnormalities in this disease. We studied a group of 484 patients with newly diagnosed symptomatic myeloma in order to better understand the prevalence of the various abnormalities and the prognostic significance of the overlapping abnormalities. A translocation involving the IgH heavy chain locus and one of the five recurrent partner chromosomes was seen in 161 (33%) patients, and 275 (57%) had trisomy of at least one odd numbered chromosome. High-risk FISH (presence of t(4;14), t(14;16), t(14;20) or loss of P53) was seen in 115 (24%) patients; median OS of this group was 3.9 years compared with not reached for standard risk patients (P<0.001). Among the patients with high-risk FISH, 49 patients who also had at least one trisomy, had a median OS that was not reached compared with 3 years for high-risk patients without a concurrent trisomy (P=0.01). Based on the current findings, we conclude that the presence of trisomies in patients with t(4;14), t(14;16), t(14;20) or p53 deletion abnormalities in myeloma ameliorates the usual adverse impact associated with these prognostic markers.
INTRODUCTION

Studies over the past decade have revealed numerous overlapping and non-overlapping genetic abnormalities in the myeloma cell and their impact on patient outcome.\textsuperscript{1-4} Given the low proliferative nature of the malignant plasma cell, conventional metaphase cytogenetics reveal the presence of karyotypic abnormalities in only a small proportion of patients.\textsuperscript{5,6} With the advent of fluorescence in situ hybridization (FISH) studies and the increasing number of probes used for the study of various abnormalities, it has become clear that nearly all patients with myeloma have one or more abnormalities that can be detected by this methodology.\textsuperscript{3,7} Currently patients with myeloma are broadly grouped into a non-hyperdiploid group with the majority of patients having a translocation involving the immunoglobulin heavy chain locus on chromosome 14 with one of the five recurrent translocation partners (on chromosomes 4, 6, 11, 16, or 20); or a hyperdiploid group.\textsuperscript{12} Hyperdiploid myeloma is typically characterized by trisomies of one or more of the odd numbered chromosomes 3, 7, 9, 11, 15, or 17. Other abnormalities such as deletions involving chromosome 1, monosomy/deletion of chromosome 17, which leads to loss of p53 gene; monosomy of chromosome 13 or interstitial deletion involving chromosome 13q; and abnormalities involving the \textit{myc} locus, are often considered to be secondary abnormalities that increase in prevalence with disease evolution. These abnormalities often overlap with each other or with any one of the primary cytogenetic abnormalities.\textsuperscript{8-12}
Prior studies have shown that abnormalities such as t(4;14), t(14;16), t(14;20) and del 17p predict for significantly shortened survival in patients with newly diagnosed disease, while hyperdiploidy has been associated with better survival.\(^3,^4,^{10,12-16}\)

However, the prognostic impact of overlapping primary cytogenetic abnormalities is unclear, especially the concurrent presence of trisomies and translocations. We studied a large group of patients with newly diagnosed myeloma who were seen at our institution, with complete FISH studies available, to address this question.

**PATIENTS AND METHODS**

We identified 500 patients with multiple myeloma, who were seen at the Mayo Clinic within 90 days of their diagnosis. Only patients who had bone marrow FISH studies performed within one year preceding their diagnosis or within 6 months following the diagnosis were included in the current study. Among this group, 16 patients did not have sufficient plasma cells observed during the FISH analysis and were excluded from the analysis. The patients received a variety of different treatments depending on the prevailing standard practice at the time of their myeloma diagnosis. A regimen containing at least one of the novel agents (thalidomide, lenalidomide or bortezomib) was used for initial therapy in 78% of the patients. The study was approved by the Mayo Clinic Institutional Review Board and was done in accordance with the Declaration of Helsinki.

**FISH Studies:** Aspirate samples were enriched for mononuclear cells using the Ficoll method and cytospin slides were prepared. FISH analysis was performed as previously described using the following probes 3cen (D3Z1), 7cen (D7Z1), 9cen
The specificity of the detection process was improved with immune-fluorescent detection of the cytoplasmic-immunoglobulin light-chain in the plasma cells as previously described (cIg-FISH). Patients were considered to have high risk disease if FISH studies demonstrated one of the following abnormalities: t(4;14), t(14;16), t(14;20), or loss of p53 gene locus (del 17p or monosomy 17) (www.msmart.org)\textsuperscript{15,17}. Patients with any of the other abnormalities or a normal FISH were considered to have standard risk multiple myeloma.

**Statistical analysis:** Fisher’s exact test was used to test differences in nominal variables. Differences in continuous variable between groups were compared using Wilcoxon signed rank test. Overall survival (OS) was defined as the time from diagnosis to death, with patients alive at the time of last follow-up censored at that date. Survival curves were constructed according to the Kaplan-Meier method and the survival curves were compared using log rank test. All analyses were performed using JMP 9.0 (SAS, Cary, NC).

**RESULTS**

The current analysis includes 484 patients diagnosed between January 1, 2004 and December 31, 2009. The median age of the patients at diagnosis was 65 (range 22-91) and 60% were male. The median estimated follow up of the entire group was 3 years (95% CI; 2.8, 3.2) from diagnosis, with 358 (74%) patients alive at the time of this analysis.
**Distribution of abnormalities:** No abnormality was found on FISH testing in 15 patients (3%), while the remaining 469 patients had one or more abnormalities. The overall frequencies of the common abnormalities are shown in Table 1. A third of the patients had one of the five recurrent translocations involving the IgH region with the common abnormalities being t(11;14), t(4;14), and t(14;16), seen in 18%, 9.5% and 5% of the patients respectively. In addition, 59 (12%) had an abnormality involving the IgH heavy chain locus that might represent a translocation involving a chromosome other than the five recurrent translocation partners or a germline abnormality (referred hence as Other IgH abnormality). Trisomy of at least one of the odd numbered chromosome (3, 7, 9, 11, 13, 15, or 17) was observed in 275 (57%) and 233 (48%) had trisomy of at least two of the odd numbered chromosomes, which is conventionally termed as hyperdiploidy. The most commonly observed trisomy was that of chromosome 9 (42%), followed by those of chromosomes 15 (37%), 11 (36%), 3 (33%), and 7 (27%). Monosomy or deletion involving chromosome 13 was seen in 228 (47%) patients, and loss of p53 gene either due to deletion involving chromosome 17p or monosomy of chromosome 17 was seen in 62 (13%) patients. In addition, we observed monosomy of chromosome 14 in 38 (8%) patients, and monosomy of chromosome 16 in 14 (3%) patients; 31 and 9 of these patients respectively also had a concurrent monosomy of chromosome 13. Finally, there were three patients with none of the above abnormalities, but had a tetraploid clone.

We then examined the overlap between the different abnormalities in individual patients and the results are as shown in Figure 1A. Since there was no overlap...
between patients with one of the five common IgH translocations and presence of other IgH abnormality, we grouped them together. Similarly, patients with trisomy of any of the odd numbered chromosomes were grouped together. Overall, 74 (15.3%) patients had a concurrent IgH translocation/abnormality and a trisomy. Monosomy 13/Del 13q was seen in 57% of patients with an IgH abnormality compared with 36% of patients with a trisomy; and 43 (9%) patients had monosomy 13/Del13q without either an IgH abnormality or trisomy. Similarly, a p53 abnormality (monosomy 17/Del17p) was seen in 14% and 11% of the groups of patients with a translocation and any trisomy, respectively with only one patient having a p53 abnormality in the absence of an IgH abnormality or trisomy. The overlap between patients with one or more of the monosomies/deletions are as shown in Figure 1B. Majority of the patients with a monosomy 14 or monosomy 16 had concurrent monosomy 13/Del 13q. In comparison, no overlap was seen between monosomy 14 and monosomy 16; and minimal overlap existed between monosomy 14 and presence of any IgH abnormality (data not shown).

**Prognostic relevance of overlapping abnormalities:** We then examined the outcome of patients based on the commonly used criteria for high-risk myeloma; presence of t(4;14), t(14;16), t(14;20) or p53 deletion (monosomy 17 or deletion 17p). High-risk FISH was seen in 115 (24%) patients, and the median OS of this group was 3.9 years (95% CI; 2.9, 5.8) compared with median not reached for the standard risk group of patients (P < 0.001) (Figure 2A). Given the observed overlap between the presence of trisomies and other types of abnormalities, and the good prognosis generally associated with hyperdiploidy, we further grouped the patients
with high-risk FISH into those with and without a concurrent trisomy of one or
more of the odd numbered chromosomes. The median OS for patients with high-risk
FISH without concurrent trisomy (N=66) was 3 years (95% CI; 2.2, 4.3) compared
with median not reached for those high-risk patients who also had a trisomy (N=49)
(P = 0.01) (Figure 2B). Conversely, when the impact of the current high-risk
stratification was examined within the group of patients with a trisomy (N=275),
there was no difference in the overall survival between the high-risk and standard-
risk groups (Figure 2C). The beneficial effect of trisomies was seen irrespective of
whether patients were classified as high risk based on the presence of p53
abnormalities or presence of the high-risk translocations (Figure 3 A-D). We also
examined if any one of the odd chromosomes had more impact compared to the
others, but did not find any such unique relationship (data not shown).

We then reclassified the patients into a new high-risk group, moving those patients
previously classified as high risk but with a trisomy into the standard risk group.
The survival of patients reclassified as high risk (66; 14%) was 36 months
compared with not reached for the standard risk (P < 0.001) (Figure 4). The hazard
ratios associated with the high-risk status was 2.2 (95% CI; 1.5, 3.2) with the older
risk stratification, and was 2.9 (95% CI; 1.9, 4.2) with the revised model.

Classification of FISH abnormalities: The current FISH classification broadly
groups patients into a hyperdiploid group and a non-hyperdiploid group that
includes all translocations and other abnormalities. Given the pattern of overlapping
abnormalities and the good prognostic effect of trisomies even in the presence of
conventional high-risk abnormalities, we reclassified the FISH abnormalities seen in myeloma into mutually exclusive groups, in terms of the common genetic abnormalities (Table 2). Based on the results of this study, we have reclassified patients into those with (i) trisomies with no concurrent IgH abnormalities, (ii) IgH locus abnormalities including translocations with the five common recurring partner chromosomes, (iii) both an IgH abnormality and trisomy, (iv) monosomy 14 without trisomy or IgH abnormalities, (v) other abnormalities, and (vi) a normal FISH. The ‘other abnormalities’ group consists primarily of patients with chromosome 13 abnormalities and loss of p53; abnormalities that are thought to have later onset and also can be seen within any of the other four categories.

DISCUSSION

The introduction of FISH testing and its routine incorporation into the evaluation of myeloma has resulted in a greater understanding of myeloma biology in the past decade.\textsuperscript{2,3,12} There is no doubt that genetic abnormalities are the main drivers of the significant heterogeneity seen in this disease both in terms of the clinical features, response to therapy, and the eventual survival outcomes. While more sophisticated and advanced technologies such as gene expression profiling using high density oligonucleotide arrays, array comparative genomic hybridization (CGH) and more recently whole genome sequencing of the tumor cells have continued to unravel the mysteries of the myeloma genome, FISH based risk classification remains the mainstay of clinical evaluation and practical approach to risk assessment.\textsuperscript{14,19,20}
Overall, the frequency of the different abnormalities in the current study is very similar to that reported by Avet-Lousseu and colleagues in a large number of myeloma patients included in the IFM99 studies, reflecting consistency with the prior reported frequencies.\(^3\) But this study makes several unique observations that have profound clinical implications in terms of risk assessment and decisions regarding therapeutic approaches, as well as for our understanding of the pathogenesis of myeloma. We show that approximately 10% of patients are in an overlapping category with presence of both IgH translocations as well as trisomies, and that this overlap has a significant impact on outcome. FISH based risk assessment has been criticized for the significant heterogeneity in outcome among high risk patients defined by the presence of t(4;14), t(14;16), t(14;20), and deletion of 17p. Previous studies have suggested that high risk features such as t(4;14) may be further classified by using other prognostic markers such as B2M or hemoglobin levels, thus explaining the heterogeneity, although this was not confirmed in a second study.\(^{21,22}\) Our results suggest that the heterogeneity in patient outcome may be explained by the presence of the overlap between different genetic abnormalities.

The findings here can also impact treatment decisions. Several studies have shown that treatment with bortezomib may improve the outcome of patients with the t(4;14) abnormality, though this has not been a uniform finding.\(^{23,24}\) It is possible that the therapeutic benefit of bortezomib may be restricted to one of the subgroups and thus may explain the heterogeneity seen between different studies. However, the relatively small number of patients in the current study and specifically the low
numbers who received bortezomib-based therapies preclude this analysis. Clearly this question merits evaluation in the larger datasets, which showed the benefit for bortezomib, such as the IFM dataset.

Hyperdiploidy has typically been considered to result from trisomies of odd numbered chromosomes and demonstrated to be a good prognostic feature.\textsuperscript{29,25} However, when examined using conventional cytogenetics or flow based ploidy evaluation trisomies of 1-3 chromosomes can often be “hidden” by the frequent presence of monosomies, especially those involving chromosomes 13 and 14. With FISH testing, patients are termed hyperdiploid only when trisomies of two or more chromosomes are observed, and trisomy indices have been developed using specific trisomies such as those involving 9, 11 and 15 to identify those with hyperdiploid myeloma.\textsuperscript{18} The result of the current study suggest that it is not necessarily the presence of “hyperdiploidy” that imparts a favorable prognosis, but rather trisomies of one or more of the odd numbered chromosomes. This raises a question as to whether we should emphasize hyperdiploidy (i.e., numeric excess on karyotype studies) or presence of trisomies as the primary pathogenetic marker that is found more often but not exclusively in the non-IgH translocated subtype of myeloma. The frequency of trisomies of different chromosomes seen in the current study is similar to that reported previously using FISH based studies.\textsuperscript{4,26} Interestingly, a good prognostic impact of trisomies was also reported previously by Perez-Simon and colleagues in the context of trisomies involving chromosomes 6 and 9.\textsuperscript{4}
The underlying mechanisms for the development of trisomies in odd numbered chromosomes in the myeloma cells as well as their favorable prognostic impact remain poorly understood. It is possible that the additional copies of these odd numbered chromosomes may increase the copy number of gene loci critical for tumor suppression, or of gene loci mediating drug sensitivity. Increased expression of genes from the trisomic chromosomes has been reported from gene expression studies, suggesting that the impact of the trisomies may be mediated through a gene dose effect. For example, the tumor suppressor genes p15 and p16 are present on 9p21 and play a role in myeloma cell proliferation due to their effect on cyclins and cyclin dependent kinases. Detailed gene expression studies of hyperdiploid patients have demonstrated subgroups within hyperdiploid MM, characterized by overexpression of certain groups of genes and with differing survival outcomes. One cannot discount the possibility that presence of trisomies is a surrogate marker for some hitherto undiscovered genetic abnormality that hampers the tumor growth in some fashion. The presence of overlapping translocations and trisomies suggests that different but non-mutually exclusive mechanisms or underlying characteristics contribute to their development. Clearly this remains an area requiring active investigation.

Given the differences in the outcome among patients with both trisomies and high risk translocations compared with those without a trisomy, we should consider classifying patients into non-overlapping categories as shown in Table 2. Currently, we classify patients with any translocation as "translocated group" irrespective of the presence of concurrent trisomies. If we take into consideration the results of
the current study, a better approach would be to group patients into those with
trisomy (ies), those with IgH abnormalities, those with both abnormalities, and
those with monosomy 14 in the absence of other abnormalities. This would still
exclude nearly 10% of patients in whom either the single abnormality is monosomy
13/Del13q and/or monosomy 17/Del17p, or have a normal FISH. The current
concept is that trisomies and IgH abnormalities are primary abnormalities present
from the earliest stages of development of the monoclonal process and monosomies
and deletions involving chromosomes 13 and 17 occur later, with increasing
prevalence during disease progression for chromosome 17 abnormalities. We have
previously shown in a temporal analysis of various abnormalities in hyperdiploid
myeloma, that trisomies are likely the earliest events followed by translocations and
monosomies/deletions.\textsuperscript{20} The results of the current study placed in the context of
this previous work suggests an evolutionary framework where trisomies develop
early on in the plasma cell evolution in over half of the patients, followed by
development of IgH translocations in nearly a half of the patients including some
who have already developed a trisomy. This is likely followed by loss of or deletions
involving chromosomes such as 13 and 17, events that continues to occur late into
the course of the disease and can occur in all the myeloma cells. Given this scenario,
isolated monosomy 13 likely represents patients with neither an IgH abnormality
nor trisomy, who acquired this abnormality with disease evolution.

In conclusion, we have further refined the category of high-risk myeloma,
identifying a group of patients with poor prognosis, and removing some of the
heterogeneity of the current stratification. In the current study we have shown for
the first time, the beneficial impact of trisomies on patients otherwise considered to have high risk myeloma, thus allowing for a more stringent definition of high risk status among myeloma patients. Our data suggests a rethinking of the hierarchy of genetic findings in terms of risk stratification, with importance given to trisomies over the translocations, reflecting a shift in the current concepts. Importantly, we are able to suggest an alternate FISH based classification in myeloma, enabling a better grouping for future that will allow more accurate comparisons across the datasets. Based on the findings of this study, we recommend that the FISH testing panel should have probes necessary to identify the presence of any one of the 5 recurrent IgH translocations, 17p deletion, trisomy of any of the odd numbered chromosomes and chromosome 13 abnormalities. Clearly these findings should be confirmed in other datasets that includes patients undergoing different types of treatment strategies, to better delineate the interaction between treatments and the presence of these abnormalities. The impact of the type of treatment on the findings here cannot be analyzed with clarity given the different types of treatments used, but a significant proportion of patients received an IMiD based regimen. It is also unclear, how the new “higher” risk patients relate to those identified as high risk using GEP. Finally, future studies need to specifically examine if the beneficial effects of therapies such as bortezomib is restricted to patients in one or the other group.
ACKNOWLEDGMENTS AND DISCLOSURES

**Author Contributions:** SK designed the study, collected and analyzed the data, and wrote the manuscript, SVR was involved in writing the manuscript, SVR, RF, MAG, MQL, AD, FKB, SRH, DD, SJR, JAL, RAK, PLB, and SRZ contributed patients and was involved in writing the manuscript, AG was involved in writing the manuscript, RPK and RK were involved with FISH testing and in writing the manuscript.

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**Disclosures:** Relevant to this work RF has received a patent for the prognostication of MM based on genetic categorization of the disease. Non-relevant; he has received consulting fees from Medtronic, Otsuka, Celgene, Genzyme, BMS and AMGEN. He also has sponsored research from Cylene and Onyx. SK has research support for clinical trials from Celgene, Millennium, Novartis, and Genzyme and is a consultant for Merck. AD and MQL received clinical trial support from Celgene.
REFERENCES


TABLE 1: Distribution of various abnormalities of FISH testing and proportion of patients with overlapping presence of trisomies

<table>
<thead>
<tr>
<th>FISH abnormality</th>
<th>Frequency N (%)</th>
<th>Trisomy also present N (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any Translocations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(11;14)</td>
<td>86 (18)</td>
<td>12 (14)</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>47 (10)</td>
<td>19 (40)</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>24 (5)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>t(6;14)</td>
<td>3 (&lt;1)</td>
<td>3 (100)</td>
</tr>
<tr>
<td>t(14;20)</td>
<td>1 (&lt;1)</td>
<td>0</td>
</tr>
<tr>
<td>Other IgH locus abnormality</td>
<td>59 (12)</td>
<td>35 (60)</td>
</tr>
<tr>
<td><strong>Any Trisomy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 chromosome</td>
<td>42 (9)</td>
<td>NA</td>
</tr>
<tr>
<td>2 chromosomes</td>
<td>52 (11)</td>
<td>NA</td>
</tr>
<tr>
<td>3 chromosomes</td>
<td>55 (11)</td>
<td>NA</td>
</tr>
<tr>
<td>4 chromosomes</td>
<td>66 (14)</td>
<td>NA</td>
</tr>
<tr>
<td>&gt;=5 chromosomes</td>
<td>60 (12)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Monosomy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monosomy 13/Del 13q</td>
<td>228 (47)</td>
<td>98 (43)</td>
</tr>
<tr>
<td>Monosomy 14</td>
<td>38 (8)</td>
<td>11 (29)</td>
</tr>
<tr>
<td>Monosomy 16</td>
<td>14 (3)</td>
<td>6 (43)</td>
</tr>
<tr>
<td><strong>P53 Abnormality</strong> (del 17p or monosomy 17)</td>
<td>62 (13)</td>
<td></td>
</tr>
<tr>
<td>Del 17p</td>
<td>49 (10)</td>
<td>26 (53)</td>
</tr>
<tr>
<td>Monosomy 17</td>
<td>13 (3)</td>
<td>5 (38)</td>
</tr>
<tr>
<td><strong>Other</strong> (all tetraploidy)</td>
<td>3 (&lt;1%)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td>15 (3%)</td>
<td>NA</td>
</tr>
</tbody>
</table>

* As a proportion of the primary abnormality
TABLE 2. Non-overlapping classification of primary molecular cytogenetic abnormalities in myeloma

<table>
<thead>
<tr>
<th>FISH abnormality</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trisomy (ies) without IgH abnormality</strong></td>
<td>201 (42%)</td>
</tr>
<tr>
<td><strong>IgH abnormality without trisomy (ies)</strong></td>
<td>146 (30%)</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>74 (18)</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>28 (10)</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>19 (5)</td>
</tr>
<tr>
<td>t(14;20)</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Unknown partner/ deletion of IgH region</td>
<td>24 (5)</td>
</tr>
<tr>
<td><strong>IgH abnormality with Trisomy (ies)</strong></td>
<td>74 (15%)</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>12 (18)</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>19 (10)</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>t(6;14)</td>
<td>3 (&lt;1)</td>
</tr>
<tr>
<td>Unknown partner/ deletion of IgH region</td>
<td>35</td>
</tr>
<tr>
<td><strong>Monosomy 14 in absence of IgH translocations or trisomy (ies)</strong></td>
<td>22 (4.5%)</td>
</tr>
<tr>
<td><strong>Other cytogenetic abnormalities in absence of IgH translocations or trisomy (ies) or monosomy 14</strong>*</td>
<td>26 (5.5%)</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td>15 (3%)</td>
</tr>
</tbody>
</table>

* These included primarily monosomy 13 and p53 abnormalities.
FIGURE LEGENDS

Figure 1: Distribution of various genetic abnormalities among patients with multiple myeloma. Panel A shows a Venn diagram demonstrating the overlapping nature between the common abnormalities seen with FISH in patients with newly diagnosed myeloma. The actual number of patients with different abnormalities is presented from among 484 patients. The remaining 19 patients either had a normal FISH (n=15) or another abnormality (n=4). Panel B shows the distribution of various monosomies/deletions and their overlap.

Figure 2: Kaplan-Meier curves demonstrating overall survival from diagnosis based on various risk factors. Panel A compares the overall survival between patients with standard risk myeloma (N=370) based on FISH testing with those with high-risk myeloma (N=114). Panel B compares the overall survival between those with standard risk myeloma (N=370), high-risk myeloma with any trisomy (N=48), and high-risk myeloma without any concurrent trisomy (N=66). Panel C compares overall survival among patients with any trisomy (N=273) with or without high-risk FISH features.

Figure 3: Kaplan-Meier curves demonstrating overall survival from diagnosis based on presence or absence of high-risk IgH translocations or P53 loss. Panels A and B compares survival between patients with high-risk IgH translocations in the absence (Panel A) or presence (Panel B) of trisomies. Panels C and D compares the overall survival between patients with P53 loss in the absence (Panel A) or presence (Panel B) of trisomies.
**Figure 4:** Survival of patients according to the revised classification: previous standard plus high-risk with trisomies (new FISH standard risk) versus high risk with no trisomies (new FISH high risk)
Figure 1A
Figure 1B
Figure 2A

![Survival analysis graph showing two curves for FISH Standard risk and FISH High risk. The graph indicates a statistically significant difference between the two groups with a P value of <0.001. The number of patients at risk at different follow-up times is shown in the legend as follows: 484, 427, 296, 178, 95, 34, and 16.]
Figure 2B

FISH Standard risk
FISH High risk with trisomy
FISH High risk

Follow up from Diagnosis (months)

Proportion surviving

Number at risk: 484 427 296 178 95 34 16

P < 0.001
Figure 4

Proportion surviving vs. follow-up from diagnosis (years)

Number at risk: 484  427  296  178  95  34  16

P < 0.001

FISH Standard risk
FISH High risk
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