Understanding the role of hyperdiploidy in myeloma prognosis: which trisomies really matter?

Marie-Lorraine Chretien,1* Jill Corre,2* Valerie Lauwers-Cances,3 Florence Magrangeas,4 Alice Cleynen,2 Edwige Yon,3 Cyrille Hulin,5 Xavier Leleu,6 Frederique Orsini-Piocelle,7 Jean-Sebastien Blade,8 Claudine Sohn,9 Lionel Karlin,10 Xavier Delbrel,11 Benjamin Hebraud,12 Murielle Roussel,12 Gerald Mari,13 Laurent Garderet,14 Mohamad Mohty,14 Philippe Rodon,15 Laurent Voillat,16 Bruno Royer,17 Arnaud Jaccard,18 Karim Belhadj,19 Jean Fontan,20 Denis Caillot,1 Anne-Marie Stoppa,21 Michel Attal,12 Thierry Facon,6 Philippe Moreau,22 Stephane Minvielle,4 Hervé Avet-Loiseau.2

*Both authors contributed equally to this work

Affiliations:

1 Department of Hematology, CHU, Dijon, France
2 Unit for Genomics in Myeloma, IUC-Oncopole; and CRCT INSERM 1037, Toulouse, France
3 Service d’Epidemiologie, CHU, Toulouse, France
4 UMGC, CHU; and INSERM U892, Nantes, France
5 Department of Hematology, CHU, Nancy, France
6 Department of Hematology, CHU, Lille, France
7 Department of Hematology, CH, Annecy, France
8 Department of Hematology, CHU, Nancy, France
9 Department of Hematology, HIA, Toulon, France
10 Department of Hematology, CH, Toulon, France
11 Department of Hematology, CH, Pau, France
12 Department of Hematology, IUC, Toulouse, France
13 Department of Hematology, CHU, Bordeaux, France
14 Department of Hematology, CHU St-Antoine, Paris, France
15 Department of Hematology, CH, Perigueux, France
16 Department of Hematology, CH, Chalon sur Saone, France
17 Department of Hematology, CHU, Amiens, France
18 Department of Hematology, CHU, Limoges, France
19 Department of Hematology, CHU, Créteil, France
20 Department of Hematology, CHU, Besançon, France
21 Department of Hematology, Institut Paoli Calmettes, Marseille, France
22 Department of Hematology, CHU, Nantes, France

**Corresponding author:** Prof Hervé AVET-LOISEAU, Unit for Genomics in Myeloma, IUC-Oncopole, 1 avenue Irène Joliot-Curie, 31059 Toulouse, France.

Phone: +33 531156142
Fax: +33 531156213
Email: avet-loiseau.h@chu-toulouse.fr
Key points

In myeloma patients, trisomy 3 improved TTP and trisomies 3 and/or 5 improved overall survival. In contrast, trisomy 21 significantly worsened overall survival.

Abstract

The prognosis of multiple myeloma is mainly dependent upon chromosomal changes. The two major abnormalities driving poor outcome are the del(17p) and the t(4;14). However, the outcome of these high-risk patients is not absolutely uniform, some patients presenting long survival. We hypothesized that these better outcomes might be related to concomitant “good risk” chromosomal changes exploring hyperdiploidy. We analyzed a large series of 965 myeloma patients including 168 patients with t(4;14) and 126 patients with del(17p) using high throughput SNP arrays after plasma cell sorting. As expected, trisomic chromosomes were highly associated. Using the LASSO model, we found that only chromosome 3 when trisomic was associated with a longer progression free survival and that three trisomies modulated overall survival (OS) in myeloma patients: Trisomies 3 and 5 significantly improved OS, and trisomy 21 worsened OS. Especially in patients with t(4;14), trisomies 3 and/or 5 seemed to overcome the poor prognosis. For the first time, using a specific modelling approach, we show that not all trisomies display the same prognostic impact. This finding could be important for routine assessment of prognosis in myeloma, some high-risk patients with a traditional evaluation could be in fact standard risk.
Introduction

In multiple myeloma (MM), prognosis is mainly dependent on the chromosomal abnormalities present in the tumor plasma cells. So far, no “good” prognosis abnormalities have been described. Some abnormalities such as hyperdiploidy, or the translocation t(11;14), are associated with a standard risk, as opposed to high-risk features which include the translocations t(4;14), t(14;16), or t(14;20), the deletion 17p (del(17p)), and the gains of the 1q arm (1q gains). The worse chromosomal abnormality is the del(17p), especially when present in the majority of the plasma cells, which is observed in 7%-8% of the patients. For instance, in the IFM experience in transplant-eligible patients, the median overall survival (OS) was 22 months. The second important chromosomal change is the translocation t(4;14), observed in 10%-15% of the patients. In the same patient population, the median OS was 41 months. The MAF translocations, i.e., t(14;16) and t(14;20), are not assessed in routine because of their low incidence, 3% and 1%, respectively, and because we did show in previous studies that the t(14;16) and (1q gains) were not independent prognostic factor. Thus, in the IFM routine practice, only del(17p) and t(4;14) are evaluated at diagnosis, and define the true high-risk patients.

Three studies did address the issue of prognostic heterogeneity in high-risk patients, with opposite conclusions. The first one concluded that concomitant trisomies did not modify the outcome of high-risk patients, whereas a second one from the same group showed that concomitant trisomies totally overcame the poor prognosis of high-risk abnormalities. A more recent study from the MRC suggested that trisomies did not modify the outcome of high-risk patients. These three studies were based on fluorescence in situ hybridization (FISH) techniques, evaluating only the presence of trisomies 3, 7, 9, and 15, or DNA content evaluation by flow cytometry. One of the main limitations of the two first studies was the relatively limited number of high-risk patients enrolled (20 and 115 patients, respectively). In the third study, the number of high-risk patients was higher, but the definition of high risk was different, including 1q gains.
In order to clearly answer to this important question, we run a very large study based on extensive genomic analysis of 965 patients analyzed by SNParray. The aims of this study was to describe the trisomies involved in myeloma, to study how they are associated with each other, to test their relationships with progression free survival and overall survival and to investigate the possibility of their influence on the effect of t(4;14) and del(17p).

Patients and Methods

We did analyze by SNP-array (Affymetrix, Santa Clara, CA), using either the SNP6.0® array, or the Cytoscan® array, depending on the time of analysis, a cohort of 965 patients diagnosed in one of the IFM center. All the patients provided signed consent for these genetic analyses, approved under the Declaration of Helsinki. Bone marrow samples were obtained at diagnosis prior treatment initiation and were shipped overnight to a central laboratory. Upon receipt, mononuclear bone marrow cells were sorted using the RoboSep system, according to manufacturer’s recommendations (StemCell Technologies, Vancouver, Canada). Only samples with more than 80% of plasma cells after sorting were kept for analysis by SNP-array. All these patients were treated in routine, and selection was based on the availability of enough frozen plasma cells, and sufficient data on clinical characteristics, treatment and follow-up. There were 56.5% of males, with a median age of 60.5 years. Regarding treatment, 191 patients received a VAD (vincristin-Adriamycin-Dexamethasone) induction followed by high-dose melphalan (HDM) and autologous stem cell transplant (ASCT) (20%), 521 patients received a VD (Velcade®-Dexamethasone) induction followed by HDM and ASCT (54%) and 26% received a non-intensive treatment because of age over 65. At the time of analysis, 60.1% of the patients were still alive. High-risk patients were defined by the presence of a del(17p) in more than 60% of the plasma cells assessed by interphase FISH on sorted plasma cells, as previously reported, and/or the presence of t(4;14) also evaluated by FISH using specific probes from Abbott (Paris, France). For hyperdiploidy, we assessed on SNP-arrays trisomies of the chromosomes, 3, 5, 7, 9, 11, 15, 17, 18, 19 and 21. Because it has been shown in childhood ALL\textsuperscript{10} and also in myeloma\textsuperscript{11} that the
number of trisomies was important for outcome (hyperdiploidy > 50 was associated with a better outcome than hyperdiploidy 47-50), we also counted the number of chromosomes on the SNP-arrays for each patient and defined hypodiploidy (< 46 chromosomes), pseudo-diploidy (46 chromosomes), mild hyperdiploidy (47 to 50 chromosomes), and large hyperdiploidy (> 50 chromosomes).

**Statistical analysis**

Pearson Chi-2 test was used to compare trisomies association between themselves, between high-risk and non high-risk myeloma and between genders. Differences in age between patients with and without a specific trisomy were compared using Student’s t-test. Time to progression (TTP) was defined as the time interval between diagnosis and progression. Overall survival (OS) was defined as the time from myeloma diagnosis until death due to any cause. Patients were censored at the date of last contact if no event was reported. Survival curves, median and inter quartile range (IQR) were obtained using the Kaplan-Meier method and comparisons between groups were made using log-rank test.

We first reported the association between each trisomy and TTP and OS using a univariate Cox model. For multivariate modelling, we included into the model t(4;14), del(17p), treatment (no ASCT versus VAD+ HDT/ASCT versus VD+ HDT/ASCT) and all the trisomies. As correlation between trisomies variables was very high (multicollinearity), the traditional multivariate Cox model produces estimates that are both biased and unstable leading to an arbitrary selection of important variables. Among the penalized regression methods developed to overcome this statistical problem we used a L1 penalized Cox regression using the LASSO (Least Absolute Shrinkage and Selection Operator) adjusted on treatment. The LASSO tends to select only few variables among a set of variables highly correlated enabling to perform variable selection and thus allows the selection of a sparser model. The regularization parameter that serves for penalization was estimated using 10 fold cross validation. The penalty term was only applied on the trisomies variables, whereas t(4;14), del(17p) and treatment were left unpenalized as they have already shown their predictive value in previous
studies. For producing a consistent selection of variables, this analysis was repeated on 500 replications obtained by bootstrapping the original data (BOLASSO). The variables selected after bootstrapping were the only ones that were non-zero (i.e., that have a predictive value) in at least 80% of the replication. Once the variables were selected, we repeated the same analysis for testing first order interactions between the selected trisomies and t(4;14), del(17p) and treatment groups, penalties were only applied to interaction effects.

In the last step, we fit a Cox model including t(4;14), del(17p), treatment and the selected trisomies adjusting further on age, sex and beta2-microglobulin level. Ultimately, we separately tested the independent effect of the two proxy variables commonly used for exploring hyperdiploidy (i.e., high hyperdiploidy and presence of at least one trisomy). The proportionality assumption was verified by evaluating Cox-Snell residuals and log-log plot. A two-sided p value lower than 0.05 was considered significant. Statistical analyses were performed on the R package “penalized” and Stata statistical software, release 11.2 (STATA Corporation, College Station, TX, USA).

Results

Translocation t(4;14) was observed in 168 patients, and del(17p) in 126 patients, 26 patients presenting both abnormalities. At least one trisomy was found in 61% of patients. The most commonly observed trisomy was chromosome 9 (48 %), followed by chromosomes 15 (47 %), 19 (46%), 5 (38%), 3 (36 %), 11 (33 %), 7 (26 %), 21 (23%) , 18 (11%) and 17 (5%). Only 13% of patients had a pseudo-diploid karyotype whereas hypodiploidy, mild hyperdiploidy, and large hyperdiploidy were observed in respectively 35%, 14% and 38% of patients. No gender difference in trisomies distribution was found except for trisomy 3 (41% vs 29%, p<0.001) and trisomy 19 (50% vs 41%, p=0.007) which were more frequent in male than female patients. Trisomy 5 was the only trisomy age related. The mean age was slightly higher in patients with a trisomy 5 than in patients without
this trisomy (61.6 years vs 59.5 years, p=0.002). The distribution of trisomies by risk groups is shown in Table 1 and associations between trisomies are reported in Figure 1.

With a median follow-up time of 4.5 years, the median TTP were 2.6 years [Inter Quartile Range(IQR) 1.5,4.5] in the standard risk group , 1.4 years [IQR 0.9,2.5] among t(4;14) patients, and 1.2 years [IQR 0.6,2.4] among del(17p) patients. According to chromosome number, median TTP were 1.8 years [0.9,3.4], 2.5 years [IQR 1.5,3.9], 2.0 years [IQR 1.1,4.2], 2.4 years [IQR 1.5,4.2] respectively for patients with hypodiploidy, pseudo-diploidy, mild hyperdiploidy and large hyperdiploidy (P=0.0005). For OS, the median survivals were 8.7 years [IQR 4.7,not reached(NR)] in the standard risk group , 3.5 years [IQR 1.6,6.8] among t(4;14) patients, and 2.7 years [IQR 1.1,4.6] among del(17p) patients. The median OS according to chromosome number were 5.0 years [2.2,9.1], 5.9 years [IQR 3.6,10.0], 5.6 years [IQR 3.0,8.2], 9.1 years [IQR 4.2, NR] respectively for patients with hypodiploidy, pseudo-diploidy, mild hyperdiploidy and large hyperdiploidy (P<0.0001). All the trisomies had a protective effect on survival in univariate analysis, except for trisomies 17, 18, and 21 which did not modify TTP or OS prognosis (Table 2).

Using the Bolasso procedure, only trisomy 3 was selected as being useful for predicting TTP (i.e., the regression coefficients were non zero in at least 400 of the 500 samples) and all others trisomies were selected in less than 50% of replications. Adjusted on age, sex, beta2 microglobulin and treatments, patients with a trisomy of chromosome 3 had a significantly improved TTP compared with patients without trisomy 3 (adjusted HR (aHR) 0.8 95% CI[0.6,0.9] p value=0.002 ; figure 2). No interaction were found between trisomy 3 and treatment group or high-risk profiles.

For predicting OS, trisomies 3, 5, and 21 were selected. Trisomies of chromosome 17, 9, 19, 15, 7, 11, 18 were non zero in 41%, 51%, 52%, 67%, 69%, 75% and 76% of replications. Adjusted on age, sex, beta2 microglobulin and treatments, patients with a trisomy of chromosome 3 (aHR 0.7 95% CI[0.5,0.9] p value=0.01) or 5 (aHR 0.7 95% CI[0.5,0.9] p value=0.02) had a significantly improved overall survival compared with patients who lacked the given trisomy whereas patients with a
trisomy of chromosome 21 (aHR 1.4 95% CI[1.0,2.0] p value=0.04) had worse outcome than patients lacking this trisomy (Table 3). Testing interactions between those trisomies and high-risk profile and treatment groups, none were reported to be consistently significant meaning that the protective effect reported for trisomy of chromosome 5 or 3 (Figure 2-3), or the risk effect reported for trisomy of chromosome 21 (Figure 2-4) was the same whatever the initial high-risk profile of patients and whatever the group of treatment. After inclusion into the model of these specific trisomies, neither large hyperdiploidy, as measured by a number of chromosomes higher than 50 (aHR 0.9 95% CI [0.5,1.7]; p value=0.813), nor at least one trisomy (aHR 1.3 95% CI [0.9,1.7]; p value=0.154) had a protective effect on overall survival. Compared to the mild hyperdiploidy group, large hyperdiploidy patients had more frequently a trisomy 3 (38.7% vs 76.1%; p<10⁻³), a trisomy 5 (32.8% vs 84.5%; p<10⁻³) and a co-occurrence of both protective trisomies (9.5% vs 64.0% p<10⁻³). Similarly trisomies 3, 5, and 3 & 5 were reported in 58.6%, 62.7% and 42.4% of patients having at least one trisomy.

Supplementary analyses were conducted for exploring the effects of the trisomies 3, 5 and 21 on deletion 1p32 (supplementary figures 1 & 2) and 1q gain (supplementary figures 3 & 4) and the results were similar to the ones presented in this report as were the survival curves including only transplanted patients (supplementary figures 5-7).

**Discussion**

Along with the International Staging System (ISS),¹⁴ chromosomal abnormalities observed in the tumor plasma cells represent the most important prognostic factor in MM. Amongst all the chromosomal changes described in MM, del(17p) and t(4;14) are the two major abnormalities which impair the survival of the patients. We did previously publish that del(17p) had an important prognostic value only if present in the major clone. This finding was recently confirmed by an
independent study. Even though the median OS of patients displaying these high-risk features is short in all the series reported in the literature, some of these patients present longer survivals. This is especially true for patients with the t(4;14). The most consistent explanation could be that other chromosomal changes may have a protective effect, even in these high-risk patients. A recent publication from the Mayo Clinic group\(^8\) did suggest that the presence of trisomies in some of these high-risk patients may improve their outcome. This study, based on the FISH technique, analyzed only trisomies 3, 7, 9, and 15 and grouped t(4;14) and del(17p) patient in the same category impairing the study of heterogeneity of survival among t(4;14) patients. Independently of the nature of the trisomy, they showed that the overall prognosis was only dependent on the presence of trisomy, totally overcoming the prognostic value of the high-risk group. This finding is of major importance for the management of the patients, meaning that trisomy assessment is mandatory for all the patients with del(17p) or t(4;14). Another recent publication from the MRC\(^9\) concluded to totally opposite findings, claiming that trisomies do not modify the outcome of high-risk patients. These apparently opposite results might be explained by several differences, especially in the definition of the high-risk group. In the MRC study, high-risk was defined by either t(4;14), del(17p), MAF translocations, or 1q gains. We did show that 1q gains cannot be considered as really a high-risk feature, with a median overall survival > 5 years.\(^5\)

Recently, more sophisticated global genomic technologies (CGH/SNP-array, next generation sequencing) have been used to assess the chromosomal changes in MM.\(^{16-18}\) They vastly confirmed the cytogenetic/FISH data, with a much higher level of definition. In a preliminary report, we did show that concomitant genetic changes worsened the outcome of high-risk patients.\(^{19}\) In order to address the issue of the role of trisomies in modulating the outcome of high-risk patients, we performed a large study based on SNP-array, in 965 patients with MM. To specifically address the issue of trisomies in high-risk patients, we enriched the series with such patients, explaining the apparently higher incidences of del(17p) and t(4;14) in this cohort of patients. This over representation of high-risk patients could have biased the HR estimates but this bias has been
studied in epidemiology and found to be very small. In this series (the largest never published on high-risk patients), we showed that trisomy 3 and/or 5 overcame the poor prognosis of t(4;14), and improved that of del(17p), whereas trisomy 21 worsened the prognostic value of both t(4;14) and del(17p). Regarding TTP, only trisomy 3 showed a statistically significant value for a better outcome.

In most of the studies exploring hyperdiploidy or trisomies in myeloma patients, proxy variables as categorization of the number of chromosome, or at least one trisomy are used to summarize the risk of a set of specific trisomies that is too large to model and to prevent high collinearity. Partitioning hyperdiploidy according to the number of chromosomes ([47-50] and [51-58]), we have clearly shown that only the category of patients with large hyperdiploidy have also a high frequency of protective trisomies. This is easily explained by the high association between trisomies. Consequently only high hyperdiploidy group can be reported has a protective marker for OS in myeloma patients because the highest the number of chromosomes, the most likely some protective trisomies will be present. This result can explain why Lim et al. had only reported a protective effect in the high hyperdiploidy group. Moreover, we also reported that only three trisomies were consistently reported as having a prognostic effect in our patients, for two of them the effect was protective and for trisomy of chromosome 21 the effect was worsening survival. This result demonstrated that a proxy of hyperdiploidy based on the presence of at least one trisomy, as reported by Kumar et al., is not in itself pertinent, first because not all trisomies are relevant, and secondly because the effects on survival probability of trisomies can be in an opposite direction.

The concordance of our results with those of Kumar, even if they did not study the same trisomies, is again explained by the high association between trisomies. In our data set, 82% of patients with a trisomy 7, 75% of patients with a trisomy 9 and 76% of patients with a trisomy 15 had also a trisomy 5. So, studying those trisomies, they have also measured the effect of trisomy 5 while this trisomy was not selected in their study. So, high hyperdiploidy or at least one trisomy can only be regarded as risk indicator and not as risk factor. Furthermore, for influencing future treatment decisions based on
cytogenetic profile of patients, we need to focus on specific risk factors and not on oversimplified markers in order to better understand the pathogenesis of myeloma and to correctly stratify patients into prognostic groups. Our work, by enlightening the greater importance of the presence of trisomies 3, 5, and 21 among all the trisomies described in myeloma patients, takes place in this framework.

The protective effect of chromosome 5 has been previously demonstrated by our group in a series of 192 patients.\textsuperscript{16} Trisomy of chromosome 3 has never been specifically studied in myeloma. How these trisomies impact the overall survival remains an unresolved question. These trisomies lead to copy number gain of hundreds of genes, preventing any target gene search. The worsening effect of trisomy 21 has never been reported previously in myeloma. This can be explained by the strategy of modelling most often used in multivariable analysis. During the first step, only variables statistically significant at a pre-specified threshold (commonly 20%) in non-adjusted analyses are included into the model. So using this strategy, it is probable that trisomy 21 had received little attention from statisticians and clinicians as the non-adjusted risk reported for trisomy 21 was almost null (HR 0.96 CI95% [0.8-1.2] P=0.748). Again, it is cumbersome to select a specific target gene responsible for this prognostic effect.

Our study is strengthened by the use of a statistical approach allowing the selection of predictive variables among highly correlated predictors, and is reinforced by bootstrapping procedures that avoid unstable selection of variables. This resampling method incorporates a small change in the data that subsequently can lead to some changes in the estimates of effect. So using bootstrapping, we controlled the stability of our results. Even though the choice of a cutoff for the proportion of bootstrap sample for which a variable is retained in order to include it in the final model is arbitrary, the choice of a higher threshold (90%) will only retain trisomy 3 for OS prognosis, and no trisomy will have been selected by choosing a stringent threshold (100%). The large sample population, the long follow-up and the SNP-array methods are also strong points that reinforced our findings.
Nevertheless, some limitations still need to be mentioned. Our study was adjusted for conventional high-risk cytogenetic factors affecting risk of death, but residual confounding due to other chromosomal abnormalities (ie gain (1q), del(1p) ) not considered in this work for the selection of trisomies may still exist. Indeed, despite the large sample studied, some groups of patients were very few represented when adding other genomic changes in our analysis. Among patients with coexistent del(17p) and del(1p32) only 8 patients had a trisomy 3 and 5 had a trisomy 5 or 21. The group sizes were even smaller for studying patients with t(4;14) and del(1p32). Moreover, this study was mainly exploratory and because we were the first to select some specific trisomies to try to explain why myeloma patients with large hyperdiploidy could have a better OS, our results need to be validated in other populations.

In conclusion, this large study found that specific trisomies could impact TTP and OS of high-risk patients. For the first time, we have shown that not all trisomies have the same prognostic effect. These results, if confirmed in others populations, could have direct consequences on the prognostic assessment modalities. For patients with high-risk features, SNP-array should be performed since presence of trisomy 3 and 5 seem to abrogate the poor outcome value of t(4;14).

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Authors contribution: MLC, JC and HAL designed the research, performed the experiments, analyzed the data and wrote the paper, VLC and EY performed the statistical analyses and wrote the paper. All other co-authors provided samples and clinical data. All of them did approve the manuscript.


Table 1: Distribution of trisomies and chromosome number according to t(4;14) and del(17p) profiles

<table>
<thead>
<tr>
<th>Trisomy</th>
<th>Standard risk group (N=633)</th>
<th>T(4;14) patients (N=168)</th>
<th>Chi-square P-value</th>
<th>Del(17p) patients (N=126)</th>
<th>Chi-square P-value</th>
<th>Chi-square P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 3</td>
<td>244 (38.5)</td>
<td>46 (27.4)</td>
<td>0.007</td>
<td>34 (27.0)</td>
<td>0.014</td>
<td>0.867</td>
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<td>Trisomy 5</td>
<td>306 (48.3)</td>
<td>15 (8.9)</td>
<td>&lt;0.001</td>
<td>26 (20.6)</td>
<td>&lt;0.001</td>
<td>0.002</td>
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<tr>
<td>Trisomy 7</td>
<td>189 (29.9)</td>
<td>18 (10.7)</td>
<td>&lt;0.001</td>
<td>23 (18.3)</td>
<td>0.008</td>
<td>0.035</td>
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<td>Trisomy 9</td>
<td>360 (56.9)</td>
<td>32 (19.0)</td>
<td>&lt;0.001</td>
<td>40 (31.7)</td>
<td>&lt;0.001</td>
<td>0.004</td>
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<tr>
<td>Trisomy 11</td>
<td>260 (41.1)</td>
<td>17 (10.1)</td>
<td>&lt;0.001</td>
<td>25 (19.8)</td>
<td>&lt;0.001</td>
<td>0.009</td>
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<td>Trisomy 15</td>
<td>336 (53.1)</td>
<td>45 (26.8)</td>
<td>&lt;0.001</td>
<td>40 (31.7)</td>
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<td>Trisomy 17</td>
<td>40 (6.3)</td>
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<td>1 (0.8)</td>
<td>0.012</td>
<td>0.061</td>
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<td>Trisomy 18</td>
<td>83 (13.1)</td>
<td>7 (4.2)</td>
<td>0.001</td>
<td>13 (10.3)</td>
<td>0.389</td>
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<td>Trisomy 19</td>
<td>335 (52.9)</td>
<td>43 (25.6)</td>
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<td>36 (28.6)</td>
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<td>Trisomy 21</td>
<td>177 (28.0)</td>
<td>17 (10.1)</td>
<td>&lt;0.001</td>
<td>18 (14.4)</td>
<td>0.001</td>
<td>0.190</td>
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<td>At least one trisomy</td>
<td>422 (66.8)</td>
<td>69 (41.1)</td>
<td>&lt;0.001</td>
<td>59 (47.2)</td>
<td>&lt;0.001</td>
<td>0.123</td>
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Chromosomes number

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<th>Standard risk group (N=633)</th>
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<th>Chi-square P-value</th>
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<th>Chi-square P-value</th>
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<td>46</td>
<td>94 (14.8)</td>
<td>17 (10.1)</td>
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<td>11 (8.7)</td>
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<td>&lt;46</td>
<td>157 (24.8)</td>
<td>102 (60.7)</td>
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<td>71 (56.3)</td>
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<td>47-50</td>
<td>82 (13.0)</td>
<td>26 (15.5)</td>
<td>&lt;0.001</td>
<td>20 (15.9)</td>
<td>&lt;0.001</td>
<td>0.468</td>
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<td>51+</td>
<td>300 (47.4)</td>
<td>23 (13.7)</td>
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<td>24 (19.0)</td>
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</tbody>
</table>

*a comparison between t(4;14) and standard risk group; ^b comparison between del(17p) and standard risk group; ^c comparison between t(4;14) and del(17p): patients with both abnormalities (n=26) were excluded from this comparison. Sixty-four patients with missing value on t(4;14) or del(17p) were excluded from this analysis.
Table 2: Unadjusted effect of trisomies and hyperdiploidy on time to progression (TTP) and overall survival (OS)

<table>
<thead>
<tr>
<th></th>
<th>TTP</th>
<th>P-value</th>
<th>OS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR [95% CI]</td>
<td></td>
<td>HR [95% CI]</td>
<td></td>
</tr>
<tr>
<td>Trisomy 3</td>
<td>0.8 [0.7-0.9]</td>
<td>&lt;0.001</td>
<td>0.6 [0.5,0.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 5</td>
<td>0.8 [0.7-0.9]</td>
<td>0.003</td>
<td>0.5 [0.4,0.7]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 7</td>
<td>0.8 [0.7-1.0]</td>
<td>0.038</td>
<td>0.8 [0.6,1.0]</td>
<td>0.030</td>
</tr>
<tr>
<td>Trisomy 9</td>
<td>0.8 [0.7-1.0]</td>
<td>0.018</td>
<td>0.6 [0.5,0.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 11</td>
<td>0.8 [0.7-0.9]</td>
<td>0.001</td>
<td>0.6 [0.4,0.7]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 15</td>
<td>0.8 [0.7-0.9]</td>
<td>0.003</td>
<td>0.6 [0.5,0.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 17</td>
<td>1.0 [0.7-1.3]</td>
<td>0.752</td>
<td>1.0 [0.6,1.5]</td>
<td>0.815</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>1.0 [0.8-1.2]</td>
<td>0.690</td>
<td>1.1 [0.8,1.5]</td>
<td>0.683</td>
</tr>
<tr>
<td>Trisomy 19</td>
<td>0.8 [0.7-1.0]</td>
<td>0.008</td>
<td>0.6 [0.5,0.8]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td>0.9 [0.8-1.1]</td>
<td>0.282</td>
<td>1.0 [0.8,1.2]</td>
<td>0.748</td>
</tr>
</tbody>
</table>

At least one trisomy

<table>
<thead>
<tr>
<th>Chromosomes number</th>
<th>TTP</th>
<th>P-value</th>
<th>OS</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&lt;46</td>
<td>1.3 [1.1-1.7]</td>
<td>0.014</td>
<td>1.5 [1.1,1.2]</td>
<td>0.014</td>
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<tr>
<td>47-50</td>
<td>1.2 [0.9-1.6]</td>
<td>0.150</td>
<td>1.4 [0.9,2.0]</td>
<td>0.113</td>
</tr>
<tr>
<td>51+</td>
<td>1.0 [0.8-1.2]</td>
<td>0.710</td>
<td>0.7 [0.5,1.0]</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Table 3: Multivariate Cox model adjusted on age, sex and beta2-microglobulin (N=767)

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>N events</th>
<th>HRa</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-HDM</td>
<td>182</td>
<td>71</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAD-HDM</td>
<td>137</td>
<td>100</td>
<td>0.78</td>
<td>[0.51;1.18]</td>
<td>0.242</td>
</tr>
<tr>
<td>VD-HDM</td>
<td>448</td>
<td>130</td>
<td>0.59</td>
<td>[0.40;0.87]</td>
<td>0.007</td>
</tr>
<tr>
<td>Translocation (4;14)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>623</td>
<td>210</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>144</td>
<td>91</td>
<td>2.20</td>
<td>[1.69;2.86]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Deletion 17p</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>676</td>
<td>232</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>91</td>
<td>69</td>
<td>3.23</td>
<td>[2.45;4.26]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trisomy 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>494</td>
<td>223</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>273</td>
<td>78</td>
<td>0.66</td>
<td>[0.48;0.91]</td>
<td>0.011</td>
</tr>
<tr>
<td>Trisomy 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>469</td>
<td>221</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>298</td>
<td>80</td>
<td>0.66</td>
<td>[0.47;0.93]</td>
<td>0.017</td>
</tr>
<tr>
<td>Trisomy 21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>584</td>
<td>235</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>183</td>
<td>66</td>
<td>1.41</td>
<td>[1.02;1.96]</td>
<td>0.040</td>
</tr>
</tbody>
</table>
Legends to Figures:

Figure 1: Graphical representation of the association between trisomies, P values are derived from a Chi-square test.

Figure 2: Kaplan-Meier estimates of time to progression according to A) t(4;14) and trisomy 3 and B) del(17p) and trisomy 3.

Figure 3: Kaplan-Meier estimates of overall survival according to A) t(4;14) and trisomy 3; B) t(4;14) and trisomy 5; C) del(17p) and trisomy 3; D) del(17p) and trisomy 5.

Figure 4: Kaplan-Meier estimates of overall survival according to A) t(4;14) and trisomy 21; B) del(17p) and trisomy 21. The curves were adjusted on trisomies 3 & 5.
Figure 1
Figure 2

A

(1) no T3, no t(4;14) (N=450)
(2) T3, no t(4;14) (N=268)
(3) no T3, t(4;14) (N=122)
(4) T3, t(4;14) (N=46)

B

(1) no T3, no deletion 17p (N=528)
(2) T3, no deletion 17p (N=310)
(3) no T3, deletion 17p (N=92)
(4) T3, deletion 17p (N=34)

P<0.0001
Figure 3

A

(1) no T3, no t(4;14) (N=450)
(2) T3, no t(4;14) (N=268)
(3) no T3, t(4;14) (N=122)
(4) T3, t(4;14) (N=46)

P<0.0001

B

(1) no T5, no t(4;14) (N=389)
(2) T5, no t(4;14) (N=329)
(3) no T5, t(4;14) (N=153)
(4) T5, t(4;14) (N=15)

P<0.0001

C

(1) no T3, no deletion 17p (N=528)
(2) T3, no deletion 17p (N=310)
(3) no T3, deletion 17p (N=92)
(4) T3, deletion 17p (N=34)

P<0.0001

D

(1) no T5, no deletion 17p (N=497)
(2) T5, no deletion 17p (N=342)
(3) no T5, deletion 17p (N=100)
(4) T5, deletion 17p (N=26)

P<0.0001

0 2 4 6 8 10 12
Years of follow-up

Survival probability (%)

0 20 40 60 80 100

Figure 4

A

- (1) no 21, no t(4;14) (N=525)
- (2) 21, no t(4;14) (N=191)
- (3) no 21, t(4;14) (N=151)
- (4) 21, t(4;14) (N=17)

B

- (1) no 21, no deletion 17p (N=631)
- (2) 21, no deletion 17p (N=207)
- (3) no 21, deletion 17p (N=107)
- (4) 21, deletion 17p (N=18)

P<0.0001
Understanding the role of hyperdiploidy in myeloma prognosis: which trisomies really matter?