The impact of clone size on the prognostic value of chromosome aberrations by fluorescence in situ hybridization in multiple myeloma

Running Title: impact of clone size on FISH in myeloma

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Authorship and Disclosures

Disclosure of Potential Conflicts of Interest

K.C.A. serves on advisory boards to Onyx, Celgene, Gilead, and Sanofi-Aventis. Y.-T.T. is a consultant to Onyx. The remaining authors declare no competing financial interests.

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Contributions

GA performed FISH, analyzed data and drafted paper. ZL analyzed data and drafted paper. QL, XQ, SY, CL and Jz helped performed FISH. YX, XF, LS, MZ, SD, WS, MH, DZ, ZZ, JQ treated the patients. YZ, CA, TC, KR, JW helped in the drafting of the paper, critical revision. K.C.A and LQ designed the research, and gave the final approval of the paper.
Translational Relevance

In contrast with leukemia, MM may have chromosomal aberrations carried by only a subset of tumor cells. The cytogenetic heterogeneity of individual cases reflected the coexistence of cytogenetically-defined aberrant plasma cell clones. It remains a matter of debate what is the prognostic significance of the size of the subclones that harbor a genetic marker, and the cutoffs used in different centers are relatively confusing. Our study indicated that the impacts of clone size on patient outcome were not equal among all forms of genetic abnormalities. Prognostic value was observed for even a subgroup of tumor plasma cells harboring the cytogenetic aberration of 13q deletion and 1q21 gains; however, 17p deletion displayed the most powerful cutoff for predicting survival only if the major clones carrying the abnormality. Our results highlighted the importance of a cytogenetic abnormality as well as the size of the subpopulation affected.
Abstract

Purpose: Accumulating evidence indicates that intratumour heterogeneity is prevalent in multiple myeloma and that a collection of multiple, genetically distinct subclones are present within the myeloma cells population. It is not clear whether the size of clonal myeloma populations harboring unique cytogenetic abnormalities carry any additional prognostic value.

Experimental Design: We analyzed the prognostic impact of cytogenetic aberrations by fluorescence in situ hybridization at different cutoff values in a cohort of 333 patients with newly diagnosed myeloma and 92 patients with relapsed myeloma.

Results: We found that nearly all IgH related arrangements were observed in a large majority of the purified plasma cells; however, 13q deletion, 17p deletion, and 1q21 amplification appeared in different percentages within the malignant plasma cell population. Based on the size of subclones carrying these cytogenetic aberrations, the patients were divided into 4 groups: 0%-10%, 10.5%-20%, 20.5%-50%, and >50%. Receiver operating characteristics analysis was applied to determine the optimal cutoff value with the greatest differential survival and showed that the most powerful clone sizes were 10% for 13q deletion, 50% for 17p deletion, and 20% for 1q21 gains, which provided the best possible cutoffs for predicting poor outcomes.

Conclusions: Our study indicated that the impact of clone size on prognostic value varies between specific genetic abnormalities. Prognostic value was observed for even a subgroup of plasma cells harboring the cytogenetic aberration of 13q deletion and 1q21 gains; however, 17p deletion displayed the most powerful cutoff for predicting survival only if the predominant clones harbored the abnormality.
**Keywords:** Multiple myeloma; Clone size; Fluorescence in situ hybridization; Cutoff value;

**Introduction**

Recent studies have indicated that intraclonal heterogeneity is a common characteristic of multiple myeloma (MM), and mixed subclones possessing different genomic alterations coexist (1). The clinical characteristics of MM are mainly determined by the dominant clones; however, mutations often existed in subclonal populations, as revealed by massively parallel sequencing (2). Moreover, minor clones have been reported to be a reservoir for relapse (3). Selective outgrowth of subclones that have a phenotypic advantage within a given tumour-microenvironmental context can result in fluctuations in subclonal architecture, and these clones later may become dominant even before the initiation of relapse treatment (4).

MM is a heterogeneous disease with the major subtypes defined by the clonal plasma cells with various genetic and epigenetic aberrations (5). Interphase FISH has emerged as the most useful clinical test for genetic aberration detection. However, the determination of cutoff selection remains a matter of debate. Although the European Myeloma Network (EMN) has recommended relatively conservative cutoff values, so far no uniform global cutoffs have been applied, and the cutoffs used in different centers are relatively inconsistent (6). For example, 17p13 deletion was prognostic only if present in at least 60% of the plasma cells in a report from IFM (7). However, in studies from Neben et al., 17p13 deletion remained the most important genetic prognostic factor in MM if it presents in 10% plasma cells (8, 9).

In contrast with leukemia, MM may have chromosomal aberrations carried by only a subset of tumor cells, and the cytogenetic heterogeneity of individual cases reflects the
coexistence of cytogenetically-defined aberrant plasma cell clones. The size of clones can be measured in a way by the percentage of cells harboring specific cytogenetic abnormalities detected by FISH. These observations raised the questions: (1) what is the prognostic significance of the size of the subclones that harbor a genetic marker; (2) what is the prognostic value if the cytogenetic aberration presents in only a fraction of the cells; and (3) what level should be set as best cutoffs to determine the most powerful prognostic significance.

To address this issue, we analyzed the prognostic impact of cytogenetic aberrations at different cutoff values in a cohort of 333 patients with newly diagnosed MM. As a control, 92 relapsed MM patients were included in this study to determine both primary genetic events and genetic progression events. Receiver operating characteristics (ROC) analysis was performed to determine the optimal clone size that predicts the greatest differential survival.
Materials and Methods

Study design

The myeloma patients included were from a prospective, nonrandomized clinical trial (BDH 2008/02) (10). The trial was done in accordance with the Declaration of Helsinki (Version 1996) and approved by the local ethics committees of institutions. We used criteria for symptomatic myeloma as defined by the International Myeloma Working Group (11). The responses were also evaluated using the International Myeloma Working Group criteria (12). A cohort of 333 consecutive patients were enrolled between January, 2004 and December, 2012, with a median follow-up time of 41 months from diagnosis.

According to their request, patients were assigned to either the thalidomide-based (arm A) or bortezomib-based (arm B) treatment. Arm A consisted of 4 cycles of induction treatment with TAD (thalidomide adriamycin, dexamethasone) or TCD (thalidomide, cyclophosphamide, dexamethasone); Arm B consisted of 4 cycles of induction treatment with BCD (bortezomib, cyclophosphamide, dexamethasone) or PAD (bortezomib, Adriamycin, dexamethasone). After at least 4 cycles of treatment with partial remission or better response, patients underwent consolidation therapy, which was either autologous stem cell transplant (ASCT) or chemotherapy with the patient’s original regimen according to their request. Subsequently, patients were treated with thalidomide (100-150 mg/day) for 1 year to maintain the response.

When relapse or progression was observed, patients would receive salvage regimen from opposite arm, as well as supportive treatment with zoledronic acid every 1-2 months and erythropoietin or granulocyte colony-stimulating factor. All patients underwent prophylactic acyclovir treatment.

FISH studies
As previously reported, all MM cell samples were purified using the Miltenyi technology (anti-CD138–coated magnetic beads) before FISH, enabling a plasma cell purity higher than 90% (13). Plasma cells were then analyzed using DNA probes (Abbott Molecular, Des Plaines, IL, USA) specific for the following chromosomal aberrations: 13q14 deletion, 17p13 deletion, t(11;14), t(4;14), and t(14;16). Gains of 1q21 were assessed using a bacterial artificial chromosome probe at 1q21 (RP11-307C12) (14). A total of 200 interphase nuclei were analyzed.

Statistical analysis

The primary end point was the correlation of cytogenetic abnormalities with survival from the time of diagnosis. Progression free survival (PFS) was calculated from the initiation of therapy to the date of death, progression, or the last follow-up. Overall survival (OS) was measured from the initiation of treatment to the date of death or last follow-up, according to the international uniform response criteria (15). Two-sided Fisher exact tests were used to assess associations between categorical variables, with a confidence coefficient of 95%. The survival curves were plotted using the Kaplan-Meier method, with differences assessed with the log-rank test. The results were considered significant if the P-value was less than or equal to 0.05.

ROC analysis was applied to determine the optimal cutoff value which yielded the greatest differential in survival. We used Youden’s index to assess the optimum cutoff point for cytogenetic abnormalities by FISH (16). The cutoff value that best discriminated (in mean of sensitivity and specificity) between survival and death was used for OS, and the cutoff value that best discriminated between progression and no progression was used for PFS.
Results

Frequencies and patterns of cytogenetic alterations

The hybridization efficiency was assessed on interphase nuclei obtained from the bone marrow (BM) of healthy donors. BM samples from 20 normal individuals were used to establish cutoff values. “Means+3×SD” of the percentages of nuclei with abnormal signals were calculated as the cutoff values for each probe. At least 200 nuclei were evaluated for each probe. The threshold levels were as follows: 13q14 deletion, 5.98%; 17p13 deletion, 3.98%; 1q21 gains, 2.15%; IgH rearrangement, 4.16%; t(11;14), 2.24%; t(4;14), 3.18%; and t(14;16), 2.76%.

By definition, an abnormality was diagnosed only when the percentage of cells with aberrant FISH signals was greater than the cutoff values. However, in order to avoid false positives, cutoff values recommended by the EMN were used: for deletions and numerical aberrations, the cutoff level was set at 20%; and for translocations in the IgH locus as well as other translocations, the cutoff level was set at 10% (17). Due to the small number of purified plasma cells in some specimens and the failure of FISH in some cases, we were unable to test the full set of probes in all patients. The exact number of probes tested and the frequency of cytogenetic abnormalities in newly diagnosed and relapsed MM are shown in Table 1. According to the percentage of plasma cells involved, patients with cytogenetic aberrations were divided into 4 groups: 0%-10%, 10.5%-20%, 20.5%-50%, and >50%. The distributions of each probes is shown in Table 2.

The frequency of malignant plasma cells in which a deletion of 13q14 was detected varied between 20-100% in newly diagnosed MM; as was true for 17p13 deletion and 1q21 gains.
Although IgH rearrangement was also detected varying between 20-100%, nearly all patients with t(11;14), t(4;14), or t(14;16) had more than 50% of plasma cells with these abnormalities (Table S1). Our results also demonstrated that the incidence of 1q21 gains, 17p deletion, and t(4;14) was higher in relapsed MM than in newly diagnosed MM; however, the frequency of 13q deletion and IgH translocation were comparable between the 2 groups (Table 1). The molecular events leading to the generation of myeloma progenitor cells are predicted to be present in all clones, whereas secondary alterations during disease progression are expected to occur in subclonal fractions. These findings support the assumption that acquisition of a chromosomal translocation into the Ig loci is a founder genetic event, whereas 17p13 deletion and 1q21 gains typically correspond to a secondary genetic change. Therefore, we focused on the cutoff selection of 13q14 deletion, 17p13 deletion, and 1q21 gains.

**Impact of 17p13 deletion on survival at different clone sizes**

Chromosome 17p13 deletion was observed in 6.6% (22/333) patients by EMN cutoff criteria. In the current study, the median percentage of plasma cells harboring 17p13 deletion was 64.5% (range: 24.5-100%). According to the percentage of plasma cells involved, patients with 17p13 deletion were divided into 4 groups: 0-10%, 10-20%, 20%-50%, and >50%. The median PFS was 24.0 (95% CI: 21.2-26.8), not reached, 10.0 (95% CI: 0-20.3), and 4.0 months (95% CI: 0-12.2), respectively, and the median OS was 52.5 (95% CI: 36.7-68.3), not reached, 32.5 (95% CI: 0-76.6), and 16.0 months (95% CI: 6.7-25.3), respectively for these four subgroups (Figure 1).

The Youden index was calculated, and cutoff points of 4.5% and 6.5% gave the highest Youden values to progression and death, respectively (Figure S1). We used 5% to group the
patients into 2 categories: their median PFS was 12.0 (95% CI: 10.1-13.9) and 24.0 months (95% CI: 21.3-26.7), respectively ($P<0.001$); while the median OS was 18.0 (95% CI: 11.5-24.5) and 53.0 months (95% CI: 36.8-69.2), respectively ($P<0.001$).

According to the survival curve, patients with del(17p) in a percentage of >50% displayed the most dismal outcome, while the other curves did not distinctly separate (Figure 1). Therefore, patients with 17p deletion were divided into 2 groups using 50% as cutoff: the median PFS of which was 4.0 (95% CI: 0-12.2) and 24.0 months (95% CI: 20.9-27.1), respectively ($P<0.001$); while the median OS was 16.0 (95% CI: 6.7-25.3) and 40.0 months (95% CI: 26.2-53.9) respectively ($P<0.001$).

**Impact of 1q21 gains on survival at different clone sizes**

Chromosome 1q21 gains were observed in 49.2% (157/319) patients by EMN criteria. In the current study, the median percentage of plasma cells exhibiting 1q21 gains was 75.5% (range: 20.5-100%). According to the percentage of plasma cells involved, patients with 1q21 gains were divided into 4 groups: 0-10%, 10-20%, 20%-50%, and >50%. The median PFS was 31.0 (95% CI: 23.9-38.0), 46.0 (95% CI: 3.4-88.6), 11.0 (95% CI: 2.2-19.8), and 14.0 months (95% CI: 9.68-18.3), respectively; and the median OS was 53.0 (95% CI: 38.6-67.3), not reached, 17.0 (95% CI: 7.9-26.1), and 24.0 months (95% CI: 20.1-27.9), respectively for these four subgroups.

Cutoff points of 20% and 25% gave the highest, but similar Youden values to progression; cutoff points of 12.7% and 22.5% gave the highest, but similar Youden values to death (Figure S1). We used 20.0% to group patients into 2 categories: the median PFS was 11.0 (95% CI: 9.9-12.1) and 23.0 months (95% CI:19.2-26.8), respectively ($P<0.001$); and the median OS
was 21.5 (95% CI: 17.5-25.5) and 52.5 months (95% CI: 29.3-75.7), respectively ($P<0.001$) (Figure 2).

**Impact of 13q14 deletion on survival at different clone sizes**

Chromosome 13q14 deletion was detected in 47.4% (158/333) patients by EMN criteria. The median percentage of plasma cells carrying the deletion was 70.5% (range: 20.5-100%). According to the percentage of plasma cells involved, patients with 13q14 deletion were divided into 4 groups: 0-10%, 10-20%, 20%-50%, and >50%; Their median PFS was 33.5 (95% CI: 24.3-42.7), 8.0 (95% CI: 0-18.3), 21.0 (95% CI: 6.1-35.9), and 16.0 months (95% CI: 12.9-19.0), respectively; while the median OS was 55.5 (95% CI: 36.8-74.2), 9.0 (95% CI: 0-21.8), 35.0 (95% CI: 18.9-51.1), and 26.0 months (95% CI: 20.9-31.1), respectively for these four subgroups.

To screen for patients likely to progress or die of MM based on 13q14, we chose a cutoff point for 13q14 deletion that gave the highest possible Youden index. A cutoff point of 10% gave the highest Youden value for both progression and death (Figure S1). The median PFS was 17.0 (95% CI: 13.8-20.2) and 33.5 months (95% CI: 24.3-42.7), respectively ($P<0.001$), and the median OS was 25.0 (95% CI: 20.1-29.9) and 55.0 months (95% CI: 36.8-74.2), respectively ($P<0.001$) (Figure 3).

In practice there was considerable concern that results just above the actual mean+3SD were artifactual, and 10% cutoff may not reflect the reality. We analyzed the patients only having 25% or more abnormal cells. We divided the patients into 3 groups: 0%-25%, 25%-50% and >50%. We found that patients with del(13q)>50% has the shortest PFS. However, regarding OS, patients with del(13q)<25% have the longest OS, and there are no differences
between 25-50% and >50% groups. Therefore, when analysis was restricted to patients having 25% or more abnormal cells, 25% is the proper cutoff value.

It is well known that the cytogenetics of myeloma are not randomly distributed, and 13q deletion is usually associated with other high-risk cytogenetic aberration, such as 17p deletion or t(4;14). We analyzed the prognostic value of del(13q) only (without t(4;14) or 17p deletion). We used three cutoff value: 10%, 20% and 50%. We found it has a slight impact on OS using 10% as cutoff value. It has no impact on PFS and OS using cutoff of 20% and 50%. It seems that the prognostic value of del(13) was almost entirely dependent on the frequent association with t(4;14) and del(17p).

**Multivariate analysis**

We then performed a multivariate analysis on all the chromosomal aberrations significantly associated with PFS and OS in the univariate analysis, as well as other parameters associated with survival in this series. Of these, 3 parameters were statistically independent predictors of PFS: chromosomal aberration 17p deletion in more than 50% plasma cells (HR 2.455 [95% CI: 1.818-3.315], \( P < 0.001 \)), 1q21 gains in more than 20% plasma cells (HR 2.401 [95% CI: 1.695-3.399], \( P < 0.001 \)), and advanced ISS stage (HR 1.377 [95% CI: 1.082-1.751], \( P = 0.009 \)). A similar analysis also identified the parameters del(17p) (HR 1.754 [95% CI: 1.291-2.383], \( P < 0.001 \)), 1q21 gains (HR 1.974 [95% CI: 1.303-2.992], \( P = 0.001 \)), and advanced ISS stage (HR 1.546 [95% CI: 1.142-2.093], \( P = 0.005 \)) as independent predictors for prediction of OS.
Discussion

The results of our study showed that there were significant differences among the sizes of clones possessing different cytogenetic aberration. Nearly all IgH-related arrangements were detected in at least 50% of purified plasma cells. However, 13q deletion, 17p deletion, and 1q21 amplification may appear in different percentages within the malignant plasma cell population of a given MM patient. The optimal clone size of 13q deletion, 17p deletion, and 1q21 amplification for prognostic value were 10%, 50%, and 20% respectively in our center.

MM develops as a result of multistep tumorigenic events characterized by frequent and complex genomic abnormalities that essentially contribute to the pathogenesis of this disease. Each step of this sequel is driven by an additional genetic event. At the top hierarchical level, cytogenetic abnormalities can be divided into primary and secondary genetic lesions. Primary genetic events occur early in the pathogenesis of MM, while secondary genomic changes are acquired with disease progression from monoclonal gammopathy of undetermined significance (MGUS) to MM and finally secondary plasma cell leukemia. By using FISH, a primary event should be detectable in nearly 100% of purified or selected plasma cells and are unlikely to change dramatically during tumor progression. However, secondary changes may only present in subpopulations and can appear with increasing frequency throughout accumulation of malignant plasma cells. These progression-related aberrations appear with a certain probability and are thus more frequent in relapsed myeloma.

Our results demonstrated that the frequency of 17p deletion and 1q21 gains was much higher in relapsed MM, but no significant differences was seen in 13q deletion and IgH translocations. The incidence of t(4;14) was also higher in relapsed MM, because patients displaying t(4;14) were at a high risk of relapse. Moreover, the frequency of malignant plasma...
cells in which a 17p deletion or 1q21 gains can be detected varied between 20-100% in newly
diagnosed MM. Although IgH rearrangement varied between 20-100%, nearly all patients with
t(11;14), t(4;14), or t(14;16) had more than 50% of plasma cells with this abnormality. IgH
rearrangement was detected using IGH dual color, break apart rearrangement probe, and
sometimes it is difficult to judge the result by assessing the separation of probe signals.
T(11;14), t(4;14), and t(14;16) were detected using dual color, dual fusion translocation probe,
allowing for objective judgment of the result. This may explain the discordance of frequency
between IgH arrangement and t(11;14), t(4;14), or t(14;16).

Our results suggested that 17p deletion and 1q21 gains were classic secondary
cytogenetic abnormalities, and that IgH related translocations were the initiating events in MM
pathogenesis. However, we were unable to judge whether 13q deletion was a primary or
secondary cytogenetic aberration. While some studies reported a similar prevalence in MGUS,
indicating that 13q deletion is an initial event in the disease, subsequent publications showed a
slightly lower prevalence, indicating that it may also be a progression event in some cases (18,
19). Our results indicated that the clone size analysis was critical for 13q14 deletion, 17p13
deletion, and 1q21 gains; however, IgH related translocations such as t(11;14), t(4;14), and
t(14;16) usually appeared in the majority of myeloma cells, and their cutoffs were proven not
critical for the analysis. We thus focused the subsequent analysis on 13q14 deletion, 17p13
deletion, and 1q21 gains.

FISH is an indispensable method to detect cytogenetic aberration in MM, since
metaphase cytogenetics is limited utility due to largely quiescent cells. FISH in myeloma has
its special features. Laboratories prefer to use their own in-house established mean+3SD from
normal BM controls as cutoff value, however, normal plasma cells represent only 0.25% of BM mononuclear cells, and these cutoff values are mainly from other cellular elements of the BM instead of plasma cells. From our experience and that of other laboratories, fluorescent signals are generally of lower intensity in the clonal PCs compared with other cellular elements of the BM (20). The decreased hybridization efficiency may result from the poor DNA accessibility for FISH probes due to abundant immunoglobulin (21). These discrepancies raise the question regarding setting accurate cutoffs in FISH experiments, especially in studies searching for deletions. How conservative the cutoffs should be remains a matter of debate, and the cutoff values are not exactly comparable among different centers. As shown in Table 3, the cutoff values are different between Arkansas, Mayo Clinic, Toronto General Hospital, Intergroupe Francophone du Myelome (IFM), University of Heidelberg, and Chinese centers.

Among all MM genetic factors, deletions of 17p remain the single most important prognostic factor, present in approximately 10% of cases at the time of diagnosis (13, 22). Deletions of 17p13 confer an inferior outcome for patients irrespective of the treatment modality used (23, 24), but the optimal cutoff remains unknown. For example, patients harboring 10-20% cells positive for 17p13 deletion are considered high-risk in Canada (25, 26) and standard-risk in Arkansas group (14, 27), using different cutoff values. The present study divided the patients with 17q deletion into 4 groups according to the percentage of plasma cells involved. Not surprisingly, patients harboring 17p13 deletion in >50% malignant plasma cells had the worst outcome. ROC methodology is routinely used to check the utility of a diagnostic marker and the best cutoff value of prognostic factors (28). Although cutoff points of 4.5% and 6.5% gave the highest Youden values to progression and death, respectively, in
practice there was considerable concern that results just above the actual mean+3×SD may not be meaningful. From the K-M curve, we also found that patients with 17p deletion in >50% malignant plasma cells had the shortest OS, while the other 3 curves were not distinctly separated. In accordance with the studies of Avet-Loiseau and Neben et al. (7, 8), the most powerful 17p13 deletion cutoffs for predicting progression-free and OS were 50%. A growing body of evidence indicates that small clones harboring 17p deletion are already present at the onset, and that selection pressure and clonal evolution cause their progressive dominance over time (29). Moreover, obligate haploinsufficiency is thought to be the mechanism underlying 17p deletion-induced tumorogenesis (30). These data may explain why the powerful prognostic value is only when 17p deletion involved the majority of the plasma cells. However, as the incidence of 17p deletion was low (<10%), and the number of patients with 17p deletion in each group was limited, this result should be validated in larger cohorts of patients.

Multiple studies have shown that chromosome 1q21 gains are associated with an inferior outcome in MM (9, 10, 31), which has been supported by several basic researches (32, 33). As in previous studies, we found that the percentage of myeloma cells carrying a 1q21 gains within total myeloma cells of a given patient increased with disease progression, indicating that 1q21 was a “progression-related” aberration. This study divided the patients with 1q21 gains into 4 groups according to the percentage of plasma cells involved. Based on the survival curve, patients were divided into 2 groups with disparate PFS and OS by a cutoff value of 20%. Moreover, the cutoff point of 20% gave the highest Youden values. Therefore, 20% may be the proper cutoff value for 1q21 gains.
Deletion of chromosome 13 is one of the most common abnormalities, found in about 50% of newly diagnosed MM patients by FISH analysis. In our study, the frequency of 13q deletion was comparable between newly diagnosed and relapsed MM. It seemed that 13q14 deletion was a primary cytogenetic aberration, but the percentage of plasma cells exhibiting del(13) varied in a wide range. There is still no consensus whether 13q deletion is a primary or secondary cytogenetic aberration. Although a cutoff point of 10% gave the highest Youden value to both progression and death, 13q14 deletion was not found to be an independent prognostic factor in the multivariate analysis, irrespective of the cutoff chosen. Actually, most of the prognostic power of 13q14 deletion was related to 17p13 deletion and 1q21 gains which are frequently associated with 13q14 deletion.

We divided the patients with 13q deletion into 4 groups according to the percentage of plasma cells involved. Unexpectedly, patients with 13q14 deletion in 10-20% of their plasma cells had the shortest OS; the OS of patients with 13q deletion in 20-50% and >50% of their plasma cells was comparable. Recent clinical and biological data are consistent with the notion that intraclonal heterogeneity is a common characteristic of MM. If a chromosomal aberration appears in ≥70% of myeloma cells, but another in only a smaller percentage of the same population (20%), there is the presence of a so-called subclonal aberration (34). Patients with 13q deletion in 10-20% of their plasma cells may possess more subclones, if other cytogenetic aberrations exist at the same time. Therefore, it is more useful to perform a panel of cytogenetics, rather than a single factor.

Taken together, our data demonstrated that, in contrast to primary IgH translocations, there is variability in the size of the abnormal clones carrying 17p deletion, 13q deletion, and
1q21 gains. The impacts of clone size on patient outcome were not equal among all forms of genetic abnormalities. In our center the 50%, 20%, and 10% were the optimal cutoff points for 17p deletion, 1q21 amplification and 13q deletion, respectively, in terms of prognostic value in this study. The prognosis was affected even by a subgroup of tumor plasma cells harboring the cytogenetic aberration of 13q deletion and 1q21 gains; however, for 17p deletion, the most powerful cutoff for predicting survival existed only when the clones become dominant. Our results highlighted the importance of a cytogenetic abnormality as well as the size of the subpopulation affected.
Reference


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

<table>
<thead>
<tr>
<th></th>
<th>Newly diagnosed MM (n=333)</th>
<th>Relapsed MM (n=92)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q deletion</td>
<td>158/333(47.4)</td>
<td>48/92(52.2)</td>
<td>0.422</td>
</tr>
<tr>
<td>1q21 gains</td>
<td>157/319(49.2)</td>
<td>63/89(70.8)</td>
<td>0.000</td>
</tr>
<tr>
<td>17p deletion</td>
<td>22/333(6.6)</td>
<td>16/92(17.4)</td>
<td>0.001</td>
</tr>
<tr>
<td>IgH rearrangement</td>
<td>203/325(62.5)</td>
<td>61/89(68.5)</td>
<td>0.276</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>73/318(23.0)</td>
<td>14/88(15.9)</td>
<td>0.154</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>71/315(22.5)</td>
<td>32/87(36.8)</td>
<td>0.007</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>13/310(4.2)</td>
<td>1/86(1.2)</td>
<td>0.178</td>
</tr>
<tr>
<td>At least one cytogenetic abnormality by FISH</td>
<td>276/323(85.4)</td>
<td>84/89(94.4)</td>
<td>0.025</td>
</tr>
<tr>
<td>High-risk cytogenetics</td>
<td>96/308(31.2)</td>
<td>43/86(50.0)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: High-risk cytogenetics were defined by the presence of t(4;14), t(14;16), and/or del(17p).
Table 2 The distribution of cytogenetics by probes.

<table>
<thead>
<tr>
<th>Chromosome aberration</th>
<th>0-10%</th>
<th>10.5-20%</th>
<th>20.5-50%</th>
<th>≥50.5%</th>
</tr>
</thead>
<tbody>
<tr>
<td>13q deletion</td>
<td>166/333(49.8)</td>
<td>9/333(2.7)</td>
<td>34/333(10.2)</td>
<td>124/333(37.3)</td>
</tr>
<tr>
<td>1q21 gains</td>
<td>149/319(46.7)</td>
<td>13/319(4.1)</td>
<td>38/319(11.9)</td>
<td>119/319(37.3)</td>
</tr>
<tr>
<td>17p deletion</td>
<td>303/333(91.0)</td>
<td>8/333(2.4)</td>
<td>7/333(2.1)</td>
<td>15/333(4.5)</td>
</tr>
<tr>
<td>IgH rearrangement</td>
<td>122/325(37.5)</td>
<td>18/325(5.5)</td>
<td>37/325(11.4)</td>
<td>148/325(45.6)</td>
</tr>
<tr>
<td>t(11;14)</td>
<td>245/318(77.0)</td>
<td>3/318(0.9)</td>
<td>5/318(1.6)</td>
<td>65/318(20.5)</td>
</tr>
<tr>
<td>t(4;14)</td>
<td>244/315(77.5)</td>
<td>3/315(1.0)</td>
<td>6/315(1.9)</td>
<td>62/315(19.6)</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>297/310(95.8)</td>
<td>0/310</td>
<td>1/310(0.3)</td>
<td>12/310(3.9)</td>
</tr>
</tbody>
</table>
Table 3 The cutoffs used in different centers.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference</th>
<th>Group</th>
<th>Methods</th>
<th>Cutoff value</th>
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</thead>
<tbody>
<tr>
<td>Neben et al. (8, 9)</td>
<td><em>Blood</em>. 2012;119(4):940-8, Haematologica. 95(7):1150-7</td>
<td>University of Heidelberg, Germany</td>
<td>MACS-FISH</td>
<td>60-70% for 17p13 deletion, 10% for 1q21 gains, 10% for 13q14 deletion, 10% for translocations</td>
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<tr>
<td>Jaksic et al. (26)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Gao et al. (43)</td>
<td><em>Med Oncol</em> 2012;29:2200-6</td>
<td>The First Affiliated Hospital of Nanjing Medical University, China</td>
<td>MACS-FISH</td>
<td>10% for fusion, 20% for numerical abnormalities</td>
</tr>
</tbody>
</table>

cig-FISH: Fluorescence in situ hybridization combined with cytoplasm immunoglobulin staining; MACS-FISH: Fluorescence in situ hybridization combined with magnetic activated cell sorting
Figure legends

**Figure1.** Impact on survival of 17p deletion at different clone sizes

**Figure2.** Impact on survival of 1q21 gains at different clone sizes

**Figure3.** Impact on survival of 13q deletion at different clone sizes
Figure 3
Clinical Cancer Research

The impact of clone size on the prognostic value of chromosome aberrations by fluorescence in situ hybridization in multiple myeloma

Gang An, Zengjun Li, Yu-Tzu Tai, et al.

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