The Recurrent IgH Translocations are Highly Associated with Non-Hyperdiploid Variant Multiple Myeloma

Rafael Fonseca MD
Carina S. Debes-Marun PhD
Elisa B. Picken
Gordon W. Dewald PhD
Sandra C. Bryant MS
Jerry M. Winkler MD
Emily Blood
Martin M. Oken MD
Rafael Santana-Dávila MD
Natalia González-Paz
Robert A. Kyle MD
Morie A. Gertz MD
Angela Dispenzieri MD
Martha Q. Lacy MD
Philip R. Greipp MD

Mayo Clinic Division of Hematology, Department of Laboratory Medicine and Pathology, Division of Biostatistics, Mayo Clinic, Rochester, Minnesota, Eastern Cooperative Oncology Group (ECOG) Statistical Center, Dana Farber Cancer Institute, Boston, MA.

Address correspondence and reprint requests to: Rafael Fonseca, MD
Consultant, Associate Professor of Medicine
Mayo Clinic Hematology/ST 6-28
Rochester, MN 55905
Tel 507-266-2040; Fax 507-266-9277
fonseca.rafael@mayo.edu

Running title (32 characters) Ploidy categories and IgH translocations

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Abstract

Aneuploid is ubiquitous in multiple myeloma (MM) and four cytogenetic subcategories are recognized; hypodiploid (associated with a shorter survival), pseudodiploid, hyperdiploid and near-tetraploid MM. The hypo, pseudo and near-tetraploid karyotypes can be referred to as the non-hyperdiploid MM. Immunoglobulin heavy-chain translocations (IgH) are seen in 60% of patients. We studied the relation between aneuploidy and IgH translocations in MM. Eighty patients with MM and abnormal metaphases were studied using interphase fluorescent in-situ hybridization (FISH) to detect IgH translocations. We also studied a second cohort of 199 patients (ECOG) for IgH translocations, chromosome 13 monosomy/deletions (∆13), and ploidy by DNA content. Mayo patients with abnormal karyotypes and FISH detected IgH translocation were more likely to be non-hyperdiploid (89% versus 39%, p<0.0001). Remarkably, 88% of patients with hypodiploidy (16/18) and 90% of patients with tetraploidy (9/10) tested had an IgH translocation. ECOG patients with IgH translocations were more likely to be non-hyperdiploid MM by DNA content (68% versus 21%, p<0.001). This association was predominantly seen in patients with recurrent chromosome partners to the IgH translocation (11q13, 4p16, and 16q23). The classification of MM into hyperdiploidy and non-hyperdiploidy is largely dictated by the recurrent (primary) IgH translocations in the latter.

Corresponding author. Rafael Fonseca fonseca.rafael@mayo.edu
Introduction

The study of chromosome abnormalities in multiple myeloma (MM) has been fraught with difficulties in elucidating an underlying order within the perceived cytogenetic chaos\(^1,2\). Abnormal metaphases are observed in only one-third of patients. This problem has prevented an accurate description of the genetic nature of MM\(^2\). While aneuploidy is common in MM, several cytogenetic subgroups can be identified based on the type of numerical chromosomal abnormalities\(^3,4\). Work done by our group and others has shown that these same subgroups are discernible by DNA content analysis (DNA flow cytometry) and by chromosome complement (karyotype analysis)\(^5-7\). Four sub-categories are readily identified hypodiploid MM, pseudodiploid MM, hyperdiploid MM and the near tetraploid MM (also call hypo-tetraploid MM)\(^7\).

Patients with either hypodiploid or pseudodiploid MM are characterized by various structural chromosomal abnormalities and monosomies. Patients with hyperdiploid MM have recurrent trisomies, particularly of chromosomes 3, 5, 7, 11, 15, 17 and 19. Near tetraploid karyotypes usually are identical duplicates of diploid or pseudodiploid karyotypes, as coexisting 2N metaphases are usually seen with the 4N component\(^6,7\). Because the cytogenetic similarities between the near-tetraploid MM karyotypes and the hypo and pseudodiploid MM, we and others have proposed two major aneuploidy groups; hyperdiploid MM (48 to 74 chromosomes) and non-hyperdiploid MM (<48 or >74 chromosomes)\(^6,7\).

The ploidy sub-categories are associated with distinct clinical presentation and outcomes. In particular hypodiploid MM is associated with a shortened survival and lower likelihood of response to therapy\(^6-10\). The prognostic significance of hypodiploid is evident when it is detected by karyotype analysis and DNA index, and is similar to that of
chromosome 13 monosomy\textsuperscript{6,7,10}. In this study we show that IgH translocations are highly associated with ploidy categories.

**Patients and Methods**

These studies were conducted under IRB approval and following the Helsinki guidelines for research with human subjects.

**Mayo Clinic Patients**

**Samples**

In total 109 patients from the Mayo Clinic were studied. We identified all patients entered into the clinical cytogenetic database at Mayo with a referral diagnosis of MM or other monoclonal gammopathy. Patients with clinical or pathological (by marrow examination or karyotype) evidence of myelodysplasia or acute leukemia were excluded. To be included in our series patients were required to have abnormal metaphases, and thus we only studied patients with MM. We initially identified 254 patients, and the overall description of these database results has been previously published by us\textsuperscript{7}. From these 254 patients we used samples from a cohort of 109 patients who gave a research bone marrow sample obtained under informed consent, and collected at the time of routine clinical procurement (Table 1).

**Bone marrow samples and karyotype analysis**

The samples for karyotype analysis of Mayo patients were obtained at the time of routine clinical procurement. The karyotypes were obtained using both short-term and long-term cultures and processed by conventional cytogenetic techniques\textsuperscript{11}. We used the
following chromosome count values to define ploidy categories; hypodiploid (up to 44 chromosomes), pseudodiploid (45 to 47 chromosomes), hyperdiploid (48 to 74 chromosomes) and near tetraploid (75 or more chromosomes).

**Stored cells and slides**

Research sample cells were enriched for the mononuclear compartment using the Ficol method. Cell cytospin slides were performed at a density of approximately $5 \times 10^5$ cells, slides were unfixed and stored at $-20\degree C$ for future use.

**ECOG patient samples**

We have studied and previously reported on 351 patients entered into the clinical trial E9486/E9487 for major cytogenetic abnormalities using interphase fluorescent *in-situ* hybridization (FISH) including; 14q32 translocations, including 14q32 without an identifiable partner, t(4;14)(p16.3;q32), t(11;14)(q13;q32), and t(14;16)(q32;q23), and Δ13-17. These patients did not have karyotype analysis routinely performed and metaphases were thus not available for analysis (not included in the database). We now report here on 199 patients in whom results were available for all the IgH translocation assays, Δ13 and ploidy determination by DNA index.

**cig-FISH studies**

Interphase FISH was performed as previously described by us using standard hybridization conditions18. Briefly we performed double color FISH and simultaneous immunofluorescent detection of the clonotypic plasma cells.
FISH probes

To detect IgH translocations we used probes that bracket this locus and as previously described by us ($V_H/C_H$ probes)\textsuperscript{19-21}. These probes are able to detect any IgH translocation via segregation of the signals (“break-apart” strategy). For the patients enrolled in the ECOG study, and reported here, we also used probes (in addition to $V_H/C_H$) to detect any of the three following most common IgH translocations using a fusion strategy; $t(4;14)(p16.3;q32)$, $t(11;14)(q13;q32)$, and $t(14;16)(q32;q23)$\textsuperscript{13-17}. 

DNA content analysis

The flow cytometry determination of total DNA content was done as previously described on the ECOG patient samples using standard staining with propidium iodide\textsuperscript{22}. This technique is better able to determine hyperdiploidy than hypodiploidy given the close proximity of the tracing for hypodiploid MM DNA peak to the pseudo-diploid peak. The following DNA index criterion were used for the determination of ploidy; <0.95 hypodiploid, 0.95 to 1.05 hyperdiploid, >1.05 hyperdiploid and <1.74 hyperdiploid and > 1.75 tetraploid.

Descriptive statistics

Descriptive statistics were used to characterize and report on the patients in this study. The Fisher’s exact test was used to test for associations between translocations and ploidy status. The Wilcoxon Rank Sum Test was used to test if a relationship existed between the presence of IgH translocation and the number of chromosomes in hypodiploid, pseudodiploid, and hyperdiploid patients. Patients were considered as having an IgH translocation if they had a positive $V_H/C_H$ assay or any one of the three specific translocations as positive by the fusion strategy.
Results

Mayo Clinic patients (n= 109)

Karyotype analysis and 14q32 translocations

Results of karyotypes and clinical and prognostic effects of translocations observed among these patients have been previously reported\(^7\). germane to this study we found that the t(11;14)(q13;q32) was observed in 29 patients. As we and others have previously demonstrated, the most frequent trisomies were those of chromosome 3, 5, 7, 9, 11, 15, 19, and 21.

Correlation between karyotypes and FISH detected IgH translocations

Eighty patients with karyotype analysis and detectable chromosome abnormalities were also studied for the presence of IgH translocations by interphase FISH (\(V_H/C_H\)). Of them 44 had evidence of an IgH translocation (55%) and 36 (45%) were normal. Patients with IgH translocations were much more likely to be non-hyperdiploid (39/44, 89%) than those without an IgH translocation (14/36, 39%) (\(p<0.0001\)) (Table 2).

Correlation between t(11;14)(q13;q32) and ploidy status

Subsequently we analyzed the 29 patients with a karyotypically detected t(11;14)(q13;q32) and found the same association (Figure 1 Aneuploidy and IgH Translocations). Of 29 patients with t(11;14)(q13;q32) 27 (93%) belonged to the non-hyperdiploid category (Table 2). Twenty-four were hypo/pseudodiploid and three were near tetraploid with 75, 80 and 83 chromosomes. Only two patients had hyperdiploidy; one with 49 chromosomes and one with 65 chromosomes. Accordingly when we analyzed all patients
(n = 109) according to the presence of any IgH translocation by either method of detection (VH/CH (n = 80) or t(11;14)(q13;q32) by karyotype (n = 29)), patients with non-hyperdiploid MM were significantly more likely to harbor an IgH translocation than patients with hyperdiploid MM (83% versus 24%, p<0.0001)(Table 2).

These same associations were detected when patients were classified according to ploidy sub-categories (Table 2). Remarkably, 16 out of 18 patients with hypodiploidy had IgH translocations (88%) and of 9 of 10 patients (90%) with near tetraploid karyotypes tested had an IgH translocation. When patients with near-tetraploid karyotype were excluded, and the chromosome number was used as a continuous variable the total chromosomal number was significantly lower in patients with IgH translocations detected (median (range), 45 (40, 66)) than for patients without IgH translocations (52 (41, 64)), p <0.0001).

**Hypodiploid MM and IgH translocations**

We further tested with cIg-FISH the 7 (of 9) patients with hypodiploid MM and IgH translocations detected by the VH/CH strategy to identify the specific IgH translocations; t(4;14)(p16.3;q32) and t(14;16)(q32;q23). Four of them had a t(4;14)(p16.3;q32) (57%), which is a higher prevalence than commonly reported for the abnormality in unselected patients (~15%).

**ECOG patients**

**Prevalence of the abnormalities**

When we extended our studies to the 199 patients entered into the ECOG E9486/E9487 clinical trial on whom we had studied also for IgH translocations, Δ13, and DNA ploidy the same results were observed (Table 3). The prevalence of Δ13 was 54% (108
of 199 patients tested), of t(11;14)(q13;q32) was 21% (41 of 197), of t(4;14)(p16.3;q32) was 16% (31 of 191) and of t(14;16)(q32;q23) it was 5% (9 of 188). In addition, another 20% of patients had an IgH translocation without an identifiable chromosome partner (40 of 199).

Among those with $\Delta 13$, 19% had a t(11;14)(q13;q32) (20 of 106), 28% had a t(4;14)(p16.3;q32), (29 of 104), and 6% had the t(14;16)(q32;q23) (6 of 103). In addition 17% of patients had an IgH translocation without an identifiable partner (81 of 108 patients with $\Delta 13$). Conversely 94% of patients with a t(4;14)(p16.3;q32) had $\Delta 13$ (29 of 31), while 67% of patients with the t(14;16)(q32;q23) had $\Delta 13$ (6 of 9), and 49% of patients with a t(11;14)(q13;q32) had $\Delta 13$ (20 of 41). Of patients with an IgH translocation without an identifiable partner 45% (18 of 40) had $\Delta 13$ too.

**Ploidy analysis by DNA content and IgH translocations**

Patients with IgH translocations were significantly more likely to be non-hyperdiploid MM than those without IgH translocations as detected by DNA content (68% versus 21%); in other words, hyperdiploid-MM patients were less likely to have IgH translocations than others (39% versus 84%) ($p<0.001$). When we compared the specific IgH translocation partners and ploidy sub-categories the same associations were observed (**Table 4**).

However, it appeared that the partners involved in the IgH translocations had a major effect on the association between ploidy and translocations. Among patients with an IgH translocation, patients with one of the three primary IgH translocations; t(4;14)(p16.3;q32), t(11;14)(q13;q32) or t(14;16)(q32;q23) were significantly more likely to be in the non-hyperdiploid category compared to those identified as having an 14q32 translocation that was not specified ($p<0.001$). As mentioned before DNA ploidy determination is not a reliable tool
for detection of hypodiploidy which explains the lower prevalence of hypodiploidy in this cohort.

**Chromosome 13 (∆13) and ploidy categories**

We have previously reported of the association between the t(4;14)(p16.3;q32) and ∆13 in MM. We wished to investigate whether the same associations with ploidy seen for the IgH translocations were present for patients with ∆13. We found that ∆13 were significantly more common in patients with non-hyperdiploid MM (72% versus 37%, p-value<0.001) (Table 5).

**Discussion**

**Summary**

Our study shows that two predominant cytogenetic subtypes of MM can be detected on the basis of the IgH translocations and total chromosomal complement; hyperdiploid MM with a low prevalence of IgH translocations, and non-hyperdiploid MM with a high prevalence of primary IgH translocations. We report these associations in two independent cohorts of patients using multiple techniques. While the associations were not absolute, there was a clear favoring for IgH translocations in the non-hyperdiploid variant MM.

We have also found that the specific partner involved in these IgH translocations is of great importance in determining the ploidy category of patients. Patients with the recurrent IgH translocations (t(4;14)(p16.3;q32), t(11;14)(q13;q32) and t(14;16)(q32;q23)) are highly favored in the non-hyperdiploid MM, while those without one of the recurrent chromosome partners do not share this association. These translocations have been proposed as “primary” by Kuehl and Bergsagel, because of the features that make them highly suspect for clonal
immortalization. In contrast, some of the translocations that do not involve recurrent partners can represent secondary genetic events that may favor clonal expansion but may not necessarily be clone initiation lesions.

We used slightly different cut-off values than have been reported, for chromosome number in the karyotypes, as we felt they better represented the underlying biology of the disease\textsuperscript{5,7}. These cut off values are only slightly different than those commonly used for the classification of other diseases such as acute leukemia. Taking into consideration the actual prevalence of IgH translocations defining pseudodiploid as those with 45 to 47 chromosomes was optimal. The associations were also present when we used the previously reported cut-off values, but the new one resulted in a better biologic classification of patients.

**Hypodiploidy and prognosis**

The association of non-hyperdiploid MM and the recurrent IgH translocations raises the possibility that the previously observed negative effects on prognosis of hypodiploid MM may be due to the high incidence of “unfavorable” IgH translocations\textsuperscript{6,7,10}. We have previously observed that the hypodiploid MM category is highly associated with Δ13 as well as chromosome 14 monosomy\textsuperscript{7}. We now show here a high incidence of t(4;14)(p16.3;q32) among a small group of patients with hypodiploid MM. We and others have shown that patients with this translocation (and we also showed this for the t(14;16)(q32;q23)) have a significantly shortened survival\textsuperscript{17,23,24}. Hypodiploid MM is the global state associated with monosomies and is highly associated with a short survival\textsuperscript{6,7,10,25}. Of interest, hypodiploidy is also associated with inferior outcome in other neoplasias\textsuperscript{26-28}.
**Hyperdiploidy and prognosis**

In contrast, it has been shown that patients with hyperdiploid MM have a more favorable outcome\textsuperscript{22,29}. In this study we have found a prevalence of hyperdiploid of 34% among the Mayo patients and 51% among the ECOG patients. These numbers are comparable to what has been published in the literature and may reflect variations in the techniques, but are not fundamentally different. We have observed similar trends among patients studied by karyotype analysis\textsuperscript{7}. This improved prognosis probably relates to the absence of any of the adverse IgH translocations. We are also aware that in other neoplasms hyperdiploidy is associated with an improved outcome\textsuperscript{30,31}, such as in childhood leukemia\textsuperscript{32}.

**Pathogenesis**

Since clonal plasma cells without IgH translocations can be detected in MGUS it is logical to hypothesize that two major pathogenetic pathways result in the generation of plasma cell neoplasms. In the first one an IgH translocation serves as the initial immortalizing event (non-hyperdiploid MM). Despite the presence of genomic instability the karyotypes of these patients is more closely related to the normal 2N or the duplicated 4N complement. The second pathway, with a genetic lesion yet to be defined, is one that favors or allows the accumulation of extra copies of chromosomes. This pathway may have unique biologic features and a similar phenotype, and may be consistent with the presence of secondary IgH translocations. For instance, in leukemias, hyperdiploidy (in the absence of other structural chromosomal aberrations) is associated with a distinct gene expression profile suggesting a common underlying pathogenetic mechanism\textsuperscript{33}. 
t(11;14)(q13;q32)

While patients with the t(11;14)(q13;q32) were initially believed to be an unfavorable category of MM\textsuperscript{34}, recent work has confirmed their improved outcome\textsuperscript{14,24}. However, since the t(11;14)(q13;q32) is represented in up to 25\% of human MM cell lines\textsuperscript{35}, it is possible that in some instances a clone with a t(11;14)(q13;q32) transforms to an aggressive phenotype after acquiring a secondary genetic “hit”, or they have less dependence overall on the bone marrow microenvironment. Such secondary genetic mechanisms could include $p16$ inactivation by promoter methylation and others\textsuperscript{36}.

Relation to the human MM cell lines

The vast majority of the human MM cell lines harbor IgH translocations\textsuperscript{37,38}. Thus, these cell lines likely originate from the non-hyperdiploid form of MM. Because of the requirements for MM cells to grow \textit{ex-vivo}, it seems reasonable to postulate that IgH translocations provide a proliferation (and perhaps survival) advantage to the clone. This advantage in turn translates into a clone that has the potential to achieve bone marrow emancipation. A possible explanation is that the transcriptional effect of the oncogenes upregulated by IgH translocations replaces the need for growth factors provided by the marrow microenvironment. This is further supported by the finding of a high incidence of IgH translocations (80\%) in the most aggressive MM variant, plasma cell leukemia. Human MM cell lines that represent hyperdiploid MM, not harboring IgH translocation, are needed to complement the current repertoire of human MM cell lines. In support of our global observations we also note that patients with plasma cell leukemia are usually non-hyperdiploid MM\textsuperscript{39-42}. When these patients are studied by DNA content they are usually non-hyperdiploid\textsuperscript{43}.
**Association of ploidy categories with Δ13**

The same associations between ploidy categories and Δ13 exist in that loss of a copy of chromosome 13 was significantly more common in patients with non-hyperdiploid variant MM. This also is consistent with previous observations made for the association between the t(4;14)(p16.3;q32) and Δ13 and also between the t(14;16)(q32;q23) and Δ13. Discerning the effects of IgH translocations versus those emanating because of the loss of a copy of chromosome 13 will be important in understanding what is the contribution for the negative prognosis of hypodiploid MM. Because the actual prevalence of Δ13 (best determined by interphase FISH) is much greater than that of t(4;14)(p16.3;q32) and t(14;16)(q32;q23), we suspect the contribution for a poor outcome in the hypodiploid MM is more likely related to these aggressive translocations and not because of the loss of chromosome 13.

**Biology of association**

There is no immediate explanation for the observed association between IgH translocations and non-hyperdiploid MM. We speculate that the survival advantage provided by IgH translocations, in the context of ongoing genomic instability, is permissive to chromosomal losses until a certain critical point (“crisis” or limit). Conversely, in patients without IgH translocations, but still in the context of genomic instability, the karyotype evolution favors hyperdiploidy and is less tolerant to chromosomal loss. This hypothesis is in need of further study, but would provide the best rationale for targeting translocations for the treatment of non-hyperdiploid MM. If the hypothesis is true, the cells may be heavily burdened by chromosomal loss that can only be sustained because of the IgH translocation proliferative signaling. This would imply that the mere down-regulation of genes involved in
IgH translocations may be sufficient to promote apoptosis or sensitize the cells to therapy agents.

**Conclusion**

The results presented here are in agreement with the published literature of chromosomes and MM. The following unifying concepts appear to be recurrent and consistent. First, MM is composed of several subgroups of patients that are best characterized by the specific genetic lesions of the clonal plasma cells. The patterns of karyotype aberrations seen in MM are not random (Figure 2). Two major subgroups of MM can be discerned; hyperdiploid MM and non-hyperdiploid MM. We now show they are closely related to the presence of the primary IgH translocations. The close association of unfavorable IgH translocations with hypodiploid variant MM is a likely a major contributor to the adverse outcome observed in these patients.
## Tables

### Table 1. Patients studied

<table>
<thead>
<tr>
<th></th>
<th>Mayo patients</th>
<th>ECOG patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Included in this study (n) *</td>
<td>109 patients *</td>
<td>199 patients</td>
</tr>
<tr>
<td>Studied for IgH translocation by cIg-FISH**</td>
<td>80</td>
<td>199 patients</td>
</tr>
<tr>
<td>Normal</td>
<td>36 (45%)</td>
<td>78 (39%)</td>
</tr>
<tr>
<td>Translocated</td>
<td>44 (55%)</td>
<td>121 (61%)</td>
</tr>
<tr>
<td>t(11;14)(q13;q32) karyotype***</td>
<td>29</td>
<td>Not applicable</td>
</tr>
<tr>
<td>DNA ploidy studies done</td>
<td>Not done</td>
<td>199 patients</td>
</tr>
</tbody>
</table>

* Includes both the 80 patients with available samples that were studied for IgH break-apart and the 29 patients with the t(11;14)(q13;q32).
** Patients were selected for further study based on the availability of samples for FISH investigation.
*** Altogether 60% of patients had an IgH translocation if we sum the 11% who had it detected by karyotype (29/254 patients) and 55% of the remaining 89% of patients.
+ All patients have been previously studied for the t(11;14)(q13;q32), t(4;14)(p16.3;q32) and t(14;16)(q32;q23).
**Table 2 IgH translocation prevalence according to non-hyperdiploid versus hyperdiploid status (Mayo patients)**

<table>
<thead>
<tr>
<th>Category</th>
<th>Status of IgH</th>
<th>n</th>
<th>Hyperdiploid</th>
<th>Non-hyperdiploid MM</th>
<th></th>
<th></th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>All patients</td>
<td>All patients</td>
<td>Hypodiploid</td>
<td>Pseudodiploid</td>
<td>Tetraploid</td>
<td></td>
</tr>
<tr>
<td><strong>V_{H}/C_{H} strategy</strong></td>
<td>Not translocated (row %)</td>
<td>36</td>
<td>22 (61%)</td>
<td>14 (39%)</td>
<td>2 (6%)</td>
<td>11 (31%)</td>
<td>1 (3%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Translocated (row %)</td>
<td>44</td>
<td>5 (11%)</td>
<td>39 (89%)</td>
<td>7 (16%)</td>
<td>26 (59%)</td>
<td>6 (14%)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>t(11;14)(q13;q32)</strong></td>
<td>All translocated (row %)</td>
<td>29</td>
<td>2 (7%)</td>
<td>27 (93%)</td>
<td>9 (31%)</td>
<td>15 (52%)</td>
<td>3 (10%)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td></td>
<td>109</td>
<td>29</td>
<td>80</td>
<td>18</td>
<td>52</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>All patients **</td>
<td>Not translocated (row %)</td>
<td>36</td>
<td>22 (61%)</td>
<td>14 (39%)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Translocated (row %)</td>
<td>73</td>
<td>7 (10%)</td>
<td>66 (90%)</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

* Numbers are skewed towards non-hyperdiploid MM, and should not be used to calculate prevalence of ploidy category because of the inclusion of karyotype detected t(11;14)(q13;q32) cases

** Includes patients with either a positive V_{H}/C_{H} break apart FISH assay or a t(11;14)(q13;q32).
Table 3. Relation between IgH translocations and DNA ploidy (ECOG patients)

<table>
<thead>
<tr>
<th>Ploidy Status</th>
<th>14q32 Translocation *</th>
<th>No 14q32 Translocation *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 121)</td>
<td>(n = 78)</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>39 (32%)</td>
<td>62 (79%)</td>
</tr>
<tr>
<td>Non-hyperdiploid</td>
<td>82 (68%)</td>
<td>16 (21%)</td>
</tr>
<tr>
<td>Hypodiploid</td>
<td>3 (2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Diploid</td>
<td>76 (63%)</td>
<td>15 (19%)</td>
</tr>
<tr>
<td>Tetraploid</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Multiple Clones</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

Fisher’s exact p-value < 0.001

* Includes t(11,14), t(4,14), t(14,16) or 14q32 translocation by V<sub>H</sub>/C<sub>H</sub> strategy.
Table 4. Ploidy sub-categories and specific IgH translocations (ECOG patients)

<table>
<thead>
<tr>
<th></th>
<th>Hyperdiploid (n = 98)</th>
<th>Non-hyperdiploid (n = 98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent (&quot;primary&quot;) IgH Translocation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t(11;14)(q13;q32)*</td>
<td>13 (16%)</td>
<td>68 (84%)</td>
</tr>
<tr>
<td></td>
<td>5 (12%)</td>
<td>36 (88%)</td>
</tr>
<tr>
<td>t(14;16)(q32;q23)</td>
<td>1 (11%)</td>
<td>8 (89%)</td>
</tr>
<tr>
<td>t(4;14)(p16.3;q32)</td>
<td>7 (23%)</td>
<td>24 (77%)</td>
</tr>
<tr>
<td>14q32 Translocation (but not one of above)</td>
<td>23 (62%)</td>
<td>14 (38%)</td>
</tr>
<tr>
<td>No IgH Translocation</td>
<td>62 (79%)</td>
<td>16 (21%)</td>
</tr>
</tbody>
</table>

p value <0.001. The P value only refers to the categories recurrent IgH ("primary") translocation versus other IgH translocations versus no IgH translocation

* The numbers in parenthesis for the specific translocations reflect the proportion of patients that were hyperdiploid or not hyperdiploid

3 patients are excluded because of unavailable results for one of the primary IgH translocations
Table 5 Relationship between chromosome 13 deletion and ploidy categories (ECOG patients)

<table>
<thead>
<tr>
<th></th>
<th>Normal 13</th>
<th>Δ13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 91 (row %)</td>
<td>n = 108 (row %)</td>
</tr>
<tr>
<td>Hyperdiploid</td>
<td>64 (70%)</td>
<td>37 (34%)</td>
</tr>
<tr>
<td>Non-hyperdiploid</td>
<td>27 (30%)</td>
<td>71 (66%)</td>
</tr>
<tr>
<td>Hypodiploid/diploid</td>
<td>26 (29%)</td>
<td>68 (63%)</td>
</tr>
<tr>
<td>Tetraploid</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Multiple Clones</td>
<td>0 (0%)</td>
<td>3 (3%)</td>
</tr>
</tbody>
</table>

p value < 0.0001
Figure Legends

Figure 1 Aneuploidy and IgH Translocations. The figure shows a colored diagram to depict the relationship between IgH translocations, numerical chromosome abnormalities and ploidy in the 109 Mayo Clinic patients studied. Each one of the columns represents a chromosome trisomy/monosomy as detected by karyotype analysis (chromosome 1 to 23 and Y from left to right). The diagram shows a trisomy (yellow) if the abnormality was present in the abnormal metaphase irrespective of the number of times present. Likewise the diagram shows a monosomy (red) if the abnormality was present in the abnormal metaphase irrespective of the number of times present. A black band in the right margin indicates that the patient has an IgH translocation and a light blue band is indicative of absence of the translocation. The left margin indicates whether the patients belong to the non-hyperdiploid variant MM (left gray bar = non-hyperdiploid MM) or the hyperdiploid variant MM (left green bar = hyperdiploid). Note the higher proportion of IgH translocations in the non-hyperdiploid group.

Figure 2 Hypothesis for the two pathogenesis pathways for MM?

According to our results, the natural hypothesis is to postulate two fundamentally separate pathways for the pathogenesis of MM. This hypothesis is based on the close relationship of ploidy category to the presence of the recurrent IgH translocations. While the majority of patients in the non-hyperdiploid group have IgH translocations patients in the hyperdiploid group have more trisomies, and they both might arise form similar clones observed at the MGUS stage. Each one of the two pathways could be independent but it is possible that in some cases the non-hyperdiploid could loose an IgH translocation
and give rise to the hyperdiploid variant MM (dashed arrow). It is currently unknown if these categories are seen since the early stages of the disease.
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Figure 1
Figure 2

Clonal Origin

Non-hyperdiploid MGUS  Non-hyperdiploid MM

\[\text{IgH Trx} \Delta 13\]  \[\Delta 13?\]  \[?\]  \[\text{IgH Trx}\]

Trisomies  \[\rightarrow\]  Trisomies

Hyperdiploid MGUS  Hyperdiploid MM
The recurrent IgH translocations are highly associated with non-hyperdiploid variant multiple myeloma

Rafael Fonseca, Carina S Debes-Marun, Elisa B Picken, Gordon W Dewald, Sandra C Bryant, Jerry M Winkler, Emily Blood, Martin M Oken, Rafael Santana-Davila, Natalia Gonzalez-Paz, Robert A Kyle, Morie A Gertz, Angela Dispenzieri, Martha Q Lacy and Philip R Greipp

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