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# JOURNAL OF CLINICAL ONCOLOGY

## REVIEWS

### HEMATOLOGIC MALIGNANCIES

Editors: *B.D. Cheson, S.J. Horning, K.C. Anderson, and H. Döhner*

**Genetics of Myeloid Malignancies:  
Pathogenetic and Clinical Implications**

*S. Fröhling et al*

**New Targeted Approaches in Chronic Myeloid Leukemia**

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**Identification and Validation of Novel Therapeutic Targets  
for Multiple Myeloma**

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# JOURNAL OF CLINICAL ONCOLOGY

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REVIEWS  
Hematologic Malignancies September 10, 2005

Guest Editors: Sandra J. Horning, MD, Kenneth C. Anderson, MD, and Harmut Döhner, MD Associate Editor: Bruce D. Cheson, MD

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## Molecular Pathogenesis and a Consequent Classification of Multiple Myeloma

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### ABSTRACT

There appear to be two pathways involved in the pathogenesis of premalignant non-immunoglobulin M (IgM) monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM). Nearly half of tumors are nonhyperdiploid, and mostly have one of five recurrent IgH translocations: 16% *11q13* (*CCN D1*), 3% *6p21* (*CCN D3*), 5% *16q23* (*MAF*), 2% *20q12* (*MAFB*), and 15% *4p16* (*FGFR3* and *MMSET*). The remaining hyperdiploid tumors have multiple trisomies involving chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, and infrequently one of these five translocations. Although cyclin D1 is not expressed by healthy lymphoid cells, it is bi-allelically dysregulated in a majority of hyperdiploid tumors. Virtually all MM and MGUS tumors have dysregulated and/or increased expression of cyclin D1, D2, or D3, providing an apparent early, unifying event in pathogenesis. The patterns of translocations and cyclin D expression (TC) define a novel classification that includes eight groups: 11q; 6p; MAF; 4p; D1 (34%); D1+D2 (6%); D2 (17%); and none (2%). The hyperdiploid D1 group is virtually absent in extramedullary MM and MM cell lines, suggesting a particularly strong dependence on interaction with the bone marrow microenvironment. Despite shared progression events (*RAS* mutations, *MYC* dysregulation, p53 mutations, and additional disruption of the retinoblastoma pathway), the phenotypes of MGUS and MM tumors in the eight TC groups is determined mainly by early oncogenic events. Similar to acute lymphocytic leukemia, MM seems to include several diseases (groups) that have differences in early or initiating events, global gene expression patterns, bone marrow dependence, clinical features, prognosis, and response to therapy.

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### INTRODUCTION

Multiple myeloma (MM), presently an incurable plasma cell (PC) malignancy with a yearly incidence of 14,000 in the United States and a median survival of 3 years, accounts for approximately 20% of deaths from hematologic malignancy and nearly 2% of deaths from cancer.<sup>1</sup> Often it is preceded by a premalignant tumor called monoclonal gammopathy of undetermined significance (MGUS), which occurs in about 3% of individuals over the age of 50.<sup>2</sup> The prevalence of both MGUS and MM increases markedly with age, and is about two-fold higher in African Americans than in whites.<sup>3</sup> Despite evidence for some clustering of MM and MGUS within

families, the roles of genetic background and environment remain unclear.<sup>4,5</sup>

### **MM Is a Plasmablast/Plasma-Cell Tumor of Post-Germinal Center B Cells**

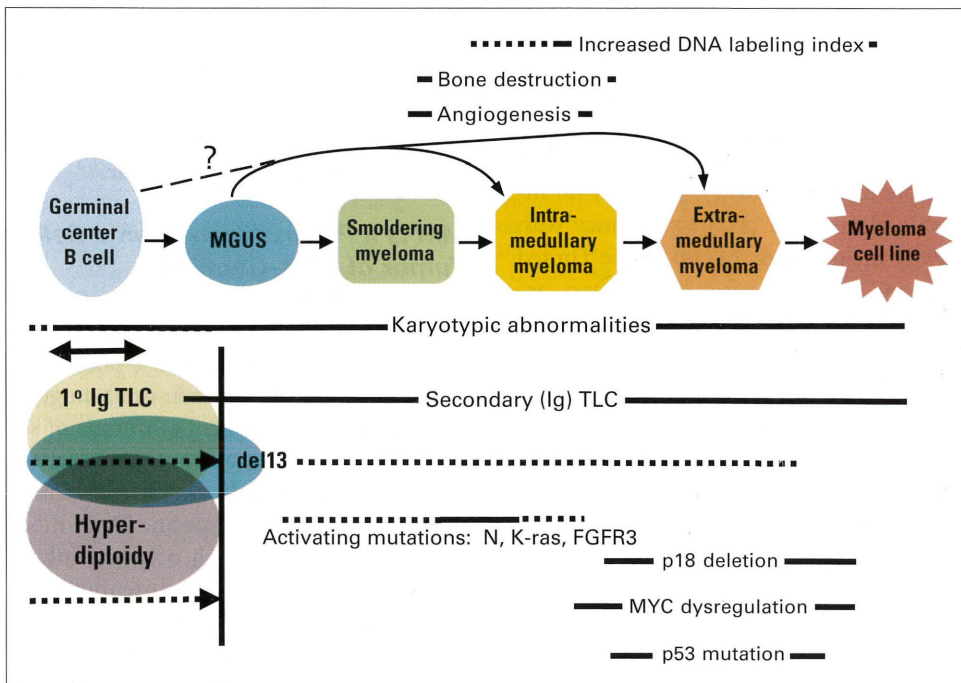
Most B-cell tumors, including MM, involve germinal center (GC) or post-GC B cells that have modified their immunoglobulin (*Ig*) genes by sequential rounds of somatic hypermutation and antigen selection, and sometimes by IgH switch recombination. These two B cell-specific DNA modification processes, which occur mainly in GC B cells, sometimes can cause mutations or double-strand DNA breaks in or near non-*Ig* genes, including oncogenes.<sup>6</sup> Post-GC B cells can generate plasmablasts

(PBs) that have successfully completed somatic hypermutation, antigen selection, and IgH switching before migrating to the bone marrow (BM), where stromal cells enable terminal differentiation into long-lived PCs.<sup>7</sup> Although PCs can be generated from pre-GC B cells, MM and non-IgM MGUS are exclusively post-GC tumors that have phenotypic features of PBs/long-lived PCs, and usually are distributed at multiple sites in the BM. A critical feature shared by MGUS and MM is an extremely low rate of proliferation, usually with no more than a small percentage of cycling cells until late stages of MM.<sup>8</sup> There appear to be two kinds of cell populations in non-IgM MGUS or MM tumors. A small fraction of proliferative tumor cells have a phenotype that is similar to a PB or a pre-PB that might express some B-cell markers (CD19, CD20, CD45) but not some PC markers (CD138), although the precise phenotype(s) and location(s) of the proliferating tumor cell remains a contentious issue.<sup>9,10</sup> However, most of the tumor cells are nonproliferative; although perhaps not fully differentiated, these cells have a phenotype that is similar to healthy, terminally differentiated, long-lived BM PCs. It is unclear if this second cell population retains the ability to revert to a proliferative phenotype. In any case, the occurrence of these two kinds of tumor cells is an important consideration in the design and evaluation of therapies.

**Stages of MM**

A clonal PC neoplasm must expand to approximately 10<sup>9</sup> cells before it produces enough Ig to be recognized as a monoclonal Ig (M-Ig) by serum electrophoresis. For

MGUS, the M-Ig is 0.5 to 3g/dl, and the tumor cells comprise no more than 10% of the mononuclear-cells in the BM (Fig 1). Depending on the level of M-Ig, 0.6% to 3% per year of patients with non-IgM MGUS progress to MM expressing the same M-Ig.<sup>11</sup> There are no unequivocal genetic or phenotypic markers that distinguish MGUS from MM tumor cells, so that it is not possible to predict if and when an MGUS tumor will progress to MM. Also, it remains unclear to what extent intrinsic genetic or epigenetic changes in the MGUS tumor cell versus extrinsic changes in non-tumor cells affect progression. Primary amyloidosis is caused by an MGUS tumor (sometimes with such a small number of tumor cells that M-Ig is not detected by serum electrophoresis) that is symptomatic because of pathologic deposits of portions of the M-Ig in critical tissues.<sup>12</sup> MM is distinguished from MGUS by having a BM tumor content > 10%.<sup>13</sup> Smoldering MM, which has a stable BM tumor content of > 10%, but no osteolytic lesions or other complications of malignant MM, has a high probability of rapidly progressing to frankly malignant MM with osteolytic lesions and/or an increasing tumor mass. Further progression of MM is associated with increasingly severe secondary features (lytic bone lesions, anemia, immunodeficiency, renal impairment), and in some patients the occurrence of tumor in extramedullary locations. Extramedullary MM is a more aggressive tumor that often is called secondary or primary plasma cell leukemia (PCL), depending on whether or not preceding intramedullary myeloma has been recognized. Human MM cell lines (HMCL) sometimes can be generated, but usually only from extramedullary tumors.



**Fig 1.** Disease stages and timing of oncogenic events. The earliest oncogenic changes are present in monoclonal gammopathy of undetermined significance (MGUS), and involve two minimally overlapping pathways (ovals), both of which substantially overlap the del 13 pathway (striped oval). Primary immunoglobulin (Ig) translocations (TLC) are thought to occur in germinal center B cells (bidirectional arrow) but the timing for the other two pathways (dashed arrows) is unclear. Other karyotypic abnormalities, including secondary (Ig) TLC may occur at all stages. Activating mutations of K- or N-RAS appear to mark, if not cause, the MGUS to multiple myeloma (MM) transition in some cases, but sometimes occur during subsequent progression of MM. Late oncogenic events that occur at a time when tumors are becoming more aggressive include MYC dysregulation by secondary (Ig) TLC, bi-allelic deletion of p18, and loss or mutation of p53.

### **Ig Translocations Are Present in a Majority of MM Tumors**

Many B-cell tumors, including MM, have chromosomal translocations that are mediated by errors in VDJ recombination or one of the other two B cell specific DNA modification mechanisms. The consequence of these translocations is dysregulation or increased expression of an oncogene that is positioned near a strong Ig enhancer. The prevalence of IgH translocations varies somewhat with the disease stage: nearly 50% in MGUS or SMM, 55% to 73% in intramedullary MM, 85% in primary PCL, and > 90% in HMCL.<sup>14-16</sup> There are five recurrent chromosomal partners (oncogenes) that are involved in IgH translocations in MGUS and MM: *4p16 (MMSET* and usually *FGFR3*), *6p21 (CCN D3)*, *11q13 (CCN D1)*, *16q23 (c-MAF)*, and *20q11 (MAFB)*.<sup>17-22</sup> Together, the combined prevalence of these five IgH translocation partners is about 40% in MM, with approximately 15% *4p16*, 3% *6p21*, 15% *11q13*, 5% *16q23*, and 2% *20q11*. The apparent markedly decreased prevalence of IgH translocations involving *4p16* and *16q23* in MGUS suggests that these translocations can cause de novo MM and/or are associated with rapid progression from MGUS to MM. The mostly simple reciprocal translocations involving the five recurrent translocation partners appear to be primary translocations that usually are mediated by errors in IgH switch recombination during the maturation of B cells in germinal centers.<sup>23</sup>

About 3% of MM tumors have secondary IgH translocations that target *c-myc* at 8q24. Secondary translocations that dysregulate a *MYC* gene (c- >> N- > L-) by juxtaposing it to an Ig locus (IgH ~ Igλ >> Igκ) or to one of many other poorly characterized chromosomal loci are late progression events.<sup>16,22,24</sup> The *MYC* translocations are absent or rare in MGUS but occur in 15% of MM tumors, 45% of advanced tumors, and 90% of HMCL. These translocations are not mediated by the B cell-specific DNA modification mechanisms, which are inactive in healthy or tumor PCs. In contrast to the primary translocations described above, these secondary events often include unbalanced and complex translocations and insertions that can involve three chromosomes, sometimes with associated amplification, duplication, inversion, or deletion.

Other IgH translocation partners have been identified in about 15% of MM tumors, and in more than 25% of MGUS tumors.<sup>14,15,22,25</sup> The other translocation partners, which are poorly characterized, appear to be mostly non-recurrent or rare. These translocations seem to share the structural complexity and lack of IgH switch region involvement observed for *MYC* translocations, suggesting that they may represent secondary translocations, which can occur at any time during tumor progression, including MGUS (A. Gabrea, unpublished data).<sup>23</sup> Translocations involving an Igλ locus occur in approximately 10% of MGUS tumors, and approximately 20% of advanced

MM tumors or HMCL.<sup>22,25</sup> Translocations involving an Igκ locus are rare, occurring in only a small percentage of MM tumors. Nearly half of IgL translocations in advanced MM tumors or HMCL target a *MYC* gene. Significantly, although all HMCL analyzed have either an IgH or IgL translocation, approximately 30% of MM tumors and 45% of MGUS tumors do not have either an IgH or IgL translocation. Surprisingly, however, two independent Ig translocations are found in 5% of MGUS tumors, 25% of advanced MM tumors, and 58% of HMCL, consistent with an accumulation of secondary Ig translocations during tumor progression (A. Gabrea, unpublished data).<sup>24</sup>

### **Hyperdiploid and Nonhyperdiploid Tumors**

All MGUS and MM tumors have numeric and/or structural chromosome abnormalities.<sup>22</sup> Although no gene has yet been identified, loss of chromosome 13/13q/13q14 sequences, which occurs in approximately 60% of MM tumors and nearly 50% of MGUS tumors, was one of the first chromosomal abnormalities associated with a poor prognosis.<sup>26,27</sup> Nearly half of MM tumors are hyperdiploid (HRD; 48-75 chromosomes), and often have multiple trisomies involving eight odd chromosomes (3,5,7,9,11,15,19,21). Nonhyperdiploid (NHRD) tumors (< 48 or > 75 chromosomes), which can be hypodiploid, pseudodiploid or subtetraploid, were noted to have a poorer prognosis than HRD tumors.<sup>28</sup> More recently it was reported that at least three of the five recurrent IgH translocations occur predominantly in NHRD tumors.<sup>29,30</sup> Secondary translocations, which appear to include all *MYC* translocations, most—if not all—IgL translocations, most IgH translocations not involving the five recurrent partners, and some IgH translocations involving the five recurrent partners, seem to occur with a similar prevalence in HRD and NHRD tumors. Loss of chromosome 13 sequences occurs in 72% of NHRD tumors but only 37% of HRD tumors. It remains to be clarified if ploidy and loss of chromosome 13 sequences are independent prognostic factors.

### **Dysregulation of cyclin D1, -2, or -3: A Unifying, Early Oncogenic Event in MM and MGUS**

Most tumor cells in MGUS and MM appear more similar to healthy, nonproliferating PCs than to healthy, but highly proliferating PBs, for which 30% or more of the cells can be in S phase. Surprisingly, however, despite a very low proliferation index, the level of cyclin D1, cyclin D2, or cyclin D3 mRNA in virtually all MM and MGUS tumors is relatively high, comparable with the level of *cyclin D2* mRNA expressed in healthy proliferating PBs, and distinctly higher than in healthy BM PCs.<sup>31</sup> Approximately 25% of MGUS or MM tumors have an IgH translocation that directly dysregulates *CCN D1 (11q13)*, *CCN D3 (6p21)*, or a *MAF* gene (*MAF*, *16q23* or *MAFB*, *20q11*) encoding a transcription factor that targets cyclin D2

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