

Multiple Myeloma: Current Perspectives

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KEYWORDS

- Multiple myeloma • Cytogenetics
- Fluorescence in situ hybridization • Monoclonal gammopathy
- Plasma cell dyscrasia • Microarray

INTRODUCTION

Multiple myeloma (MM) is a rarely curable malignancy defined by a proliferation of monoclonal plasma cells (PCs) in the bone marrow (BM), often with an elevated serum or urine paraprotein (M protein, or monoclonal protein). It is the second most common hematologic cancer in the United States and Europe, with an incidence of 20,000 or more cases per year,^{1,2} representing 1% of all cancers and 2% of all cancer deaths. MM is primarily a disease of the elderly (median age at diagnosis, 67 years) with only approximately 3% of patients diagnosed before the age of 40. The cause of MM remains unknown, but associated risk factors include obesity and a family history of lymphoid hematopoietic cancers. The disease is more prevalent in men than in women and is highest in blacks and lowest in Asians.³

MM is heterogeneous at many levels, including clinical presentation, pathogenetic characteristics, response to treatment, and outcome. MM emerges through a multistep transformation from one or two asymptomatic, premalignant phases—monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM).^{4,5} Patients with symptomatic MM present with one or more features of CRAB, an acronym for hypercalcemia, renal insufficiency, anemia, and bone lesions.⁶ The International Myeloma Working Group laboratory criteria for the different stages of MM are shown in **Table 1**.⁶

MGUS, with a reported incidence of about 3% per year in people aged older than 60 years, and the more advanced premalignant stage of SMM share the same primary genetic aberrations detected in MM. They are not treated, however, because of the low likelihood of progression and the unknown effects of early intervention. The risk of progression to MM is about 1% per year for MGUS patients and about 10% per year for the first 5 years for SMM patients. Many patients remain under observation for years before transformation occurs.^{7,8} There is also no supporting evidence that early intervention in asymptomatic disease will improve overall survival (OS) or quality

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Table 1 Multiple myeloma categories proposed by the International Myeloma Working Group				
Category	Criteria	Treatment	Risk of MM Progression per Year	Comments
MGUS	Serum M protein ^a <3 g/dL Clonal bone marrow plasma cells <10% No evidence of related organ or tissue impairment (CRAB ^b)	None Observation and close follow-up are recommended	~1%	Progresses to MM in ~20% of patients
SMM	Serum M protein ≥3 g/dL or clonal bone marrow plasma cells ≥10% or both No evidence of related organ or tissue impairment (CRAB)	Treatment (within clinical trials only) Bisphosphonates given for bone loss	~10%	
MM	≥10% clonal bone marrow plasma cells Serum or urine M protein (except in nonsecretory MM ^c) Related organ or tissue impairment (CRAB)	Immediate treatment Clinical trials Bisphosphonates for patient with bone lesions		Aggressive relapses occur in some genetic subtypes ^d

^a M protein, or monoclonal protein, is the abnormal immunoglobulin produced by myeloma cells.

^b CRAB is an acronym for the major clinical features of myeloma—hypercalcemia (serum Ca ≥11.5 mg/dL), renal insufficiency (serum creatinine >2 mg/dL), anemia (>2 g/dL below normal), and bone lesions.

^c Nonsecretory myeloma requires ≥10% clonal plasma cells; no M protein is detectable by serum/urine electrophoresis or elevated κ or λ light chains by the free light chain assay; 1% to ~2% of patients present with nonsecretory MM.

^d Aggressive relapse is characterized by acute renal failure, circulating plasma cells, deep cytopenias, extramedullary plasmacytomas (malignant plasma cells that localize to bone or soft tissue), and resistance to conventional anti-MM therapies.

of life, lead to unexpected complications such as secondary myeloid neoplasia, or result in an earlier onset or more aggressive disease.⁸ MM, in contrast, with destruction of bone and suppression of bone marrow function, among other symptoms, requires immediate treatment.

The critical event in MM pathogenesis is thought to be the transformation of a proliferative “plasmablastic” cell located in the lymphoid germinal centers.^{9,10} The progeny of this “stem cell” migrate to the bone marrow where they mature to terminally differentiated antibody-secreting PCs. Acquired cytogenetic and molecular alterations associated with malignant transformation of PCs dysregulate the normal developmental pathway, leading to altered cell behavior. Based on the premise that hyperdiploidy or an aberrant *IGH* gene rearrangement leading to cyclin D dysregulation is an early, disease-defining event in the transformation process,^{9,11,12} Gonzalez and colleagues⁹ compared serial samples collected from patients in transition from MGUS to MM or at relapse and found no intraclonal diversity. These data suggest that from the precursor states of MGUS and SMM to MM, the abnormal plasma cell clone carries the same primary chromosomal aberration (*IGH* translocations or hyperdiploidy) and secretes the same paraprotein for the duration of the disease.^{8,9} Fulminant MM and its progression are typically associated with the presence of additional karyotypic aberrations, shorter therapeutic responses, and shortened OS.

Heterogeneity of disease course and outcome shows a striking relationship with the genetic alterations of the malignant PCs. Traditionally, MM has been associated with a poor prognosis and an OS of 3 to 7 years from diagnosis, although the range has varied from less than 1 year in patients with aggressive disease to greater than 10 years in patients with indolent disease. But the management of patients with MM has changed dramatically over the past decade. Emerging new therapies, such as thalidomide, lenalidomide, and bortezomib, have increased the median OS to 4 to 5 years and a 20% chance of surviving to greater than 10 years.^{13–15} Despite these achievements, MM is usually considered incurable. Refinement of the current genetically defined risk groups is needed to identify high-risk patients who may benefit from novel treatments from patients with predictable clinical courses who benefit from existing treatments. To address this need, the International Myeloma Working Group recently proposed consensus guidelines for the workup of patients with suspected MM using standardized diagnostic, prognostic, and response criteria.^{6,16–18} Currently, patient stratification is based on the known pathogenetic factors of age; tumor burden; renal function; serum levels of lactic dehydrogenase (LDH); β_2 -microglobulin ($S\beta_2M$); and albumin, plasma cell labeling index, functional status, and cytogenetics (Table 2). This stratification model will soon be strengthened with the incorporation of oncogenomic diagnostic techniques including analysis of DNA copy number alteration by array comparative genomic hybridization (aCGH), single nucleotide polymorphism (SNP) microarrays, and gene expression profiling (GEP).^{18–20} This article reviews the conventional cytogenetics (CC), molecular cytogenetics, and genomic diagnostics of MM and highlights a few recent clinical trials that demonstrate the impact of genetic risk stratification on the treatment of this plasma cell malignancy.

HISTORICAL PERSPECTIVE OF CYTOGENETICS IN MM

Dewald and colleagues were the first to describe the clinical utility of chromosome studies in MM.²¹ In their seminal paper, they showed that compared with patients with karyotypically normal bone marrow cells, patients with karyotypically abnormal

Table 2 International Myeloma Working Group risk stratification guidelines for multiple myeloma	
Minimal recommendations for risk stratification	Additional studies suggested for optimal risk stratification
Serum albumin and β_2 -microglobulin assays	Conventional (metaphase) cytogenetics
Bone marrow examination with FISH to detect t(4;14), t(14;16), and del(17p) in PCs ^{a,b}	Labeling index
Lactic acid dehydrogenase levels	MRI/PET imaging
Immunoglobulin type	Microarrays (CGH/SNP) to determine DNA copy number alterations
Histology to determine plasmablastic disease	Gene expression profiling

^a Identified by plasma cell specific FISH protocols.

^b Genetic testing in MM clinical trials may also include: (1) FISH testing for deletion 1p and 1q gains, (2) DNA copy number alterations by CGH/SNP microarrays, or (3) gene expression profiling, or a combination of these.

bone marrow cells had reduced OS, underscoring the relevance of the plasma cell genome to disease outcome. Normal PCs are terminally differentiated cells with low proliferative activity. The presence of karyotypically normal bone marrow studies in MM patients provides clear evidence of active, normal hematopoiesis, whereas patients with abnormal karyotypes show a higher proliferative, less differentiated PC neoplasm indicative of active disease and higher tumor burden. Over the following 15 years, conventional cytogenetic studies reported an incidence of abnormal PC karyotypes in 30% to approximately 40% of newly diagnosed and 35% to approximately 60% of previously treated MM patients.^{22–27} Despite the relatively low abnormality rate, the data exposed two clinically significant findings: (1) conventional cytogenetic studies are a surrogate for the plasma cell proliferative index assay, with either test capable of indicating active proliferating disease in the bone marrow; and (2) prognostic differences are associated with ploidy level and with specific recurring cytogenetic aberrations (**Fig. 1**).

Because the low proliferative potential of terminally differentiated PCs limits detection of chromosomal aberrations by CC, which requires metaphases, interphase fluorescence in situ hybridization (I-FISH) has become an attractive technique to interrogate MM-specific chromosomal aberrations. All patients with abnormal karyotypes show known abnormalities by I-FISH, but the two assays provide slightly different information. I-FISH detects specific prognostically significant cytogenetic markers in malignant PCs, in particular, high-risk *IGH* translocations such as t(4;14)/*IGH/FGFR3* and t(14;16)/*IGH/MAF* that are not obvious by CC because they are cryptic or hidden in complex karyotypes.²⁸ However, I-FISH detects only its intended target and provides no information about additional abnormalities that may be present or may signal disease progression. Metaphase cytogenetics, however, is an excellent tool for detecting the wide range of karyotypic alterations observed in MM and, as mentioned previously, serves as a surrogate index for active proliferative disease. Thus, I-FISH is not a substitute for CC; it has clear clinical utility when CC is either noninformative or not interpretable and is a necessary adjunct to metaphase cytogenetics for detecting subtle *IGH* translocations. In addition, both interphase and metaphase

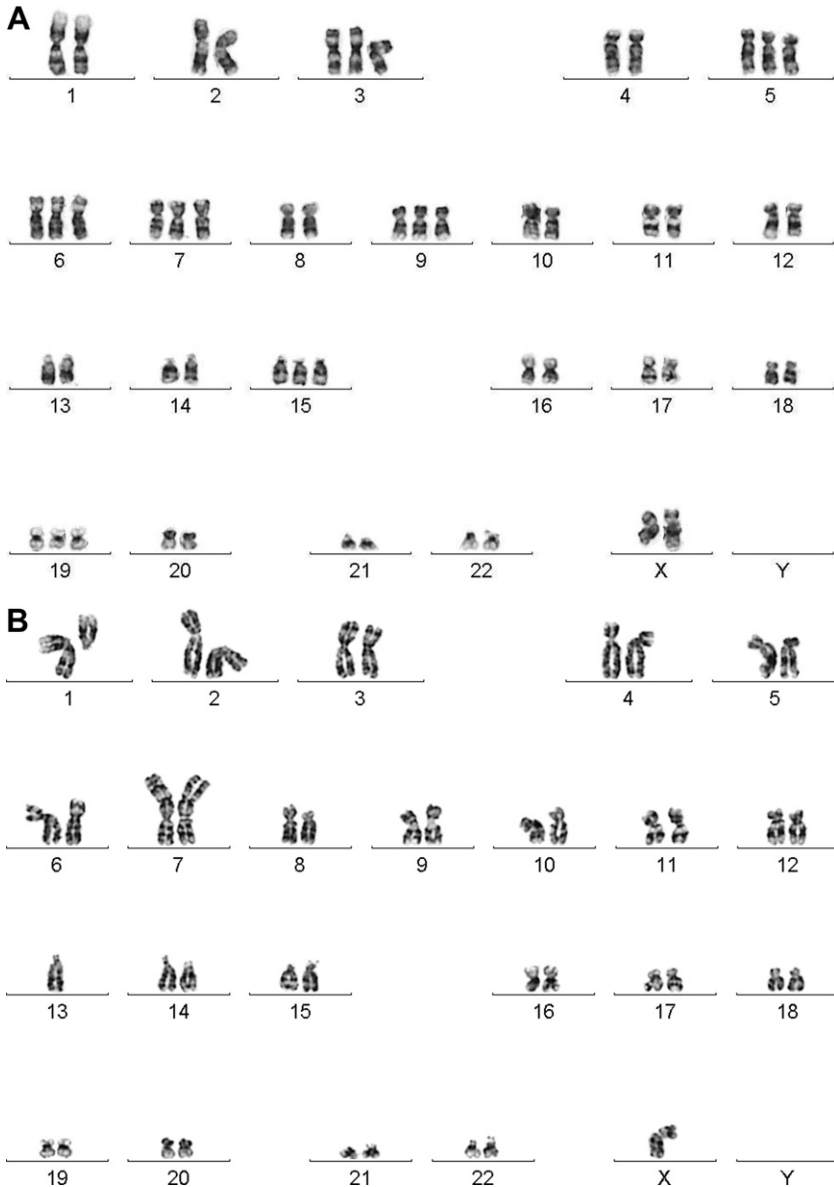


Fig. 1. Representative abnormal karyotypes found in MM. (A) Hyperdiploid karyotype (53 chromosomes) showing gain of the typical "odd number" chromosomes. (B) Hypodiploid karyotype (44 chromosomes) showing loss of chromosome Y, monosomy 13, gain of 1q by unbalanced rearrangements involving chromosome 7, and deletion of 8p and 11q. A "cryptic" *IGH* gene rearrangement was also detected by FISH (not shown).

FISH studies extend the advantage that the residual cell pellet from metaphase studies or the G-banded slides can be used to refine data gleaned from the CC study. Together, CC and FISH (both metaphase and interphase) play important and independent roles in MM stratification by providing timely and prognostically

relevant information for newly diagnosed patients and by defining secondary aberrations associated with clonal evolution.

CC Methods

The tissue of choice for the study of MM is BM collected at presentation. A small aliquot of a BM aspirate is cultured for 24 to approximately 72 hours and a minimum of 20 metaphase cells from two or more cultures is analyzed. Some laboratories report a higher yield of metaphase cells if they add B-cell cytokines or growth factors such as interleukin-4 (IL4) or IL6 to the medium.

A comprehensive analysis includes G-banded karyotyping and metaphase FISH, when applicable. With metaphase cytogenetics alone, a laboratory can expect informative results in approximately 40% of cases, but metaphase FISH is required to show cryptic or hidden *IGH* fusions and deletions in approximately 10% of cases.²⁸ Periodic follow-up cytogenetic studies should be recommended to detect clonal evolution of disease or evolving therapy-related myeloid neoplasms.

FISH Studies

Dewald and colleagues²⁸ reported that bone marrow aspirates from 54% of newly diagnosed MM patients with normal CC showed clonal anomalies by I-FISH. However, when BM samples contained 20% or more PCs, the percentage of detected anomalies by I-FISH rose to approximately 85%. A Mayo Clinic/Eastern Cooperative Oncology Group (ECOG) study using a FISH assay combined with an immunoglobulin (clg) assay that stains PC cytoplasmic Ig light chains detected 60% *IGH* translocations compared with 11% detected by karyotyping, with all 11% showing t(11;14)(q13;q32) (**Fig. 2**).²⁹ Based on those findings, the International Myeloma Working Group discourages the use of FISH on unselected BM cells and recommends the use of plasma cell-specific FISH.⁶ Using CC and PC FISH, laboratories can expect to find a PC clone in more than 90% of newly diagnosed MM patients.^{19,28}

Identifying PCs for FISH analysis may be accomplished in any of three ways: (1) magnetic-activated cell sorting using anti-CD138 microbeads (purity approximately 90%)^{19,30–35}; (2) clg-FISH³⁶; or (3) morphology using sequential May–Grünwald Giemsa staining to identify malignant PCs by their characteristic morphology, followed by FISH (**Fig. 3A–J**).³⁷ The last technique has the advantage of correlating cytogenetic aberrations with cell lineage. Correlating discrepant CC results with cell morphology allows for the detection of myeloid neoplasms coexisting with MM (see **Fig. 3K,L**).

The Mayo Clinic and the International Myeloma Working Group recommend the following FISH probes for risk stratification: –13/del(13q), del(17p)/TP53, and the two *IGH* high-risk translocations that are not clearly visible by CC, t(4;14)(p16;q32) and t(14;16)(q32;q23) (**Fig. 4**; see **Table 2**).^{15,18} Because hyperdiploidy and t(11;14)(q13;q32) are the most common aberrations observed in MM and define the standard risk group, many clinical laboratories test for them routinely.

Other Molecular Assays

Information on copy number alterations in bone marrow samples can be detected with array comparative genomic hybridization (aCGH) or high-resolution single nucleotide polymorphism (SNP) arrays. These DNA-based arrays are highly effective in determining the chromosomal site, size, and genetic content of copy number alterations and defining the minimal region of gain or loss. Together with GEP, mutation analysis, and methylation studies, these genomic tools have the potential to

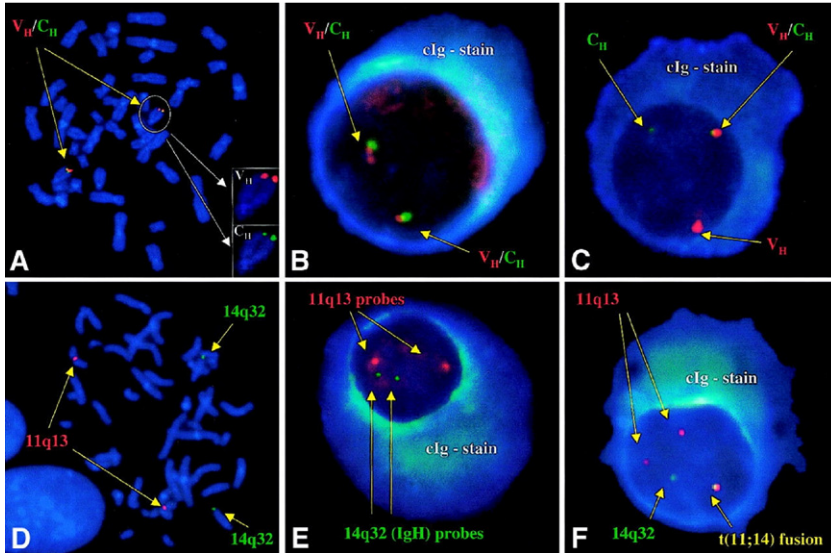


Fig. 2. Normal and abnormal clg FISH examples in plasma cell dyscrasia. (A) Normal hybridization pattern of the variable heavy chain (V_H) and constant heavy chain (C_H) probes of the immunoglobulin heavy chain (*IGH*) gene localize to their locus on chromosome 14 at band 14q32 on a normal male metaphase cell. (B) Normal *IGH* pattern with the V_H - C_H probes closely paired. Note the intense blue fluorescence of the cytoplasmic light chain (clg). (C) 14q32/*IGH* translocation detected by separation of one V_H - C_H pair using a break-apart probe set. (D) A normal hybridization pattern using the t(11;14)(q13;q32) or *IGH/CCND1* probe set on a normal male metaphase cell. No fusions are seen. Probes localized to their normal sites. (E) Normal hybridization pattern for t(11;14) probe set in a plasma cell. No fusions are present. (F) A fusion is present in this plasma cell indicating the presence of a t(11;14) or *CCND1/IGH* gene rearrangement. (Reprinted from Hayman SR, Bailey RJ, Jalal SM, et al. Translocations involving the immunoglobulin heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. *Blood* 98:2266–8, 2001 [Fig. 1]; with permission.)

identify candidate genes important in myeloma pathogenesis and to establish a new molecular risk stratification model. As with PC-FISH, these molecular assays yield the best results when purified PCs are used.^{18,30–35,38,39}

CLINICAL SIGNIFICANCE OF CYTOGENETIC ABERRATIONS

The first broad classification of MM is based on ploidy. Hyperdiploidy and non-hyperdiploidy represent the two major pathways for MGUS progression to MM (Table 3; see Fig. 1).^{7,11,24,29,40–45} The hyperdiploid subgroup comprises approximately 60% of MM patients and demonstrates better OS than the non-hyperdiploid subgroup. Key findings in the hyperdiploid subgroup include karyotypes with 48 to 74 chromosomes, frequent gains of odd-numbered chromosomes (especially 3, 5, 7, 9, 11, 15, 19, or 21), and compared with the non-hyperdiploid subgroup, less frequent structural aberrations and less frequent *IGH* translocations (~10%–20%). Using high-resolution SNP arrays, the rank order of frequency of whole chromosomes gained in hyperdiploid MM is 15 > 9 > 5 > 19 > 3 > 11 > 7 > 21.³³ In the non-hyperdiploid subtype, in contrast, the 40% of MM patients with generally poor OS share chromosome counts of 47 or less or 75 or greater and numerous structural

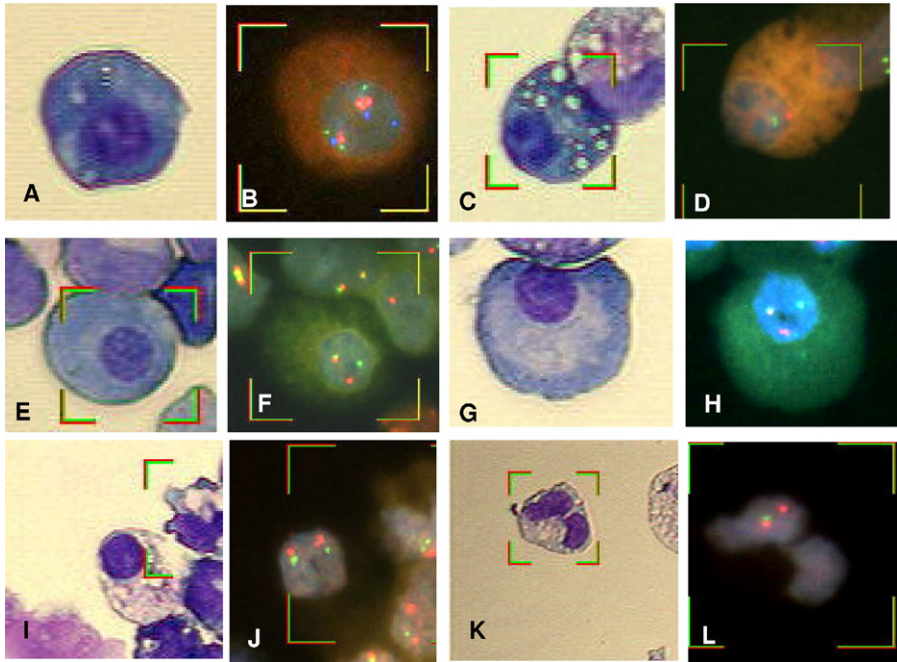


Fig. 3. Examples of a sequential morphology/FISH technique using May–Grünwald Giemsa to identify PCs by their characteristic morphology followed by FISH to detected specific MM aberrations. The plasma cell in (A) shows gain of chromosomes 5, 9, and 15 (hyperdiploid) in frame (B). The plasma cell in (C) shows a monosomy 13 hybridization pattern in (D). The plasma cell in (E) shows an *IGH* gene rearrangement using a break-apart probe set (F). The plasma cell in (G) shows a fusion in (H) indicating the presence of the derivative chromosome 4 using a t(4;14) probe set. The red and green signals in this plasma cell denote the normal chromosomes 4 and 14; the derivative chromosome 14 is not present in this PC. The plasma cell in (I) shows a normal chromosome 13 hybridization in (J); however, a myeloid cell (segmented neutrophil) in (K) from the same patient sample shows a hybridization pattern consistent with deletion 13q, indicating the presence of a coexisting myelodysplastic disorder. (Modified from Slovak ML, Bedell V, Pagel K, et al. Targeting plasma cells improves detection of cytogenetic aberrations in multiple myeloma: phenotype/genotype fluorescence in situ hybridization. *Cancer Genet Cytogenet* 2005;158:99–109; with permission).

aberrations, including frequent *IGH* translocations (~70%).^{11,29,43–47} Chromosomes 13, 14, 16, and 22 are frequently lost in hypodiploid (defined as less than 45 chromosomes) MM. Near-tetraploid karyotypes are included in the non-hyperdiploid group because they appear to be doubling products of pseudodiploid or hypodiploid karyotypes.^{24,29,43,48}

***IGH* Translocations Are Primary Aberrations in MM**

IGH translocations targeting the *IGH* class switching region are reported in approximately 65% of patients with MM.^{29,42,49} Because *IGH* translocations are detected in MGUS and SMM, they are considered the primary “immortalizing” events, yet alone they are not sufficient for transformation.¹¹ At the molecular level, these translocations juxtapose oncogenes into the proximity of the *IGH*

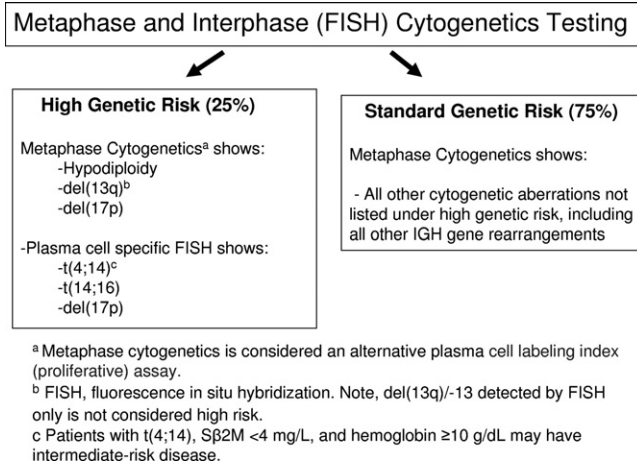


Fig. 4. Genetic testing in newly diagnosed multiple myeloma for risk stratification. (Adapted from Fig. 1 from Kumar SK, Mikhael JR, Buadi FK, et al. Management of newly diagnosed symptomatic multiple myeloma: updated Mayo Stratification of Myeloma and Risk-Adapted Therapy [mSMART] consensus guidelines. Mayo Clin Proc 2009;84:1095–110; with permission.)

enhancers, driving abnormal expression of the translocated oncogenes. Each translocation subgroup is associated with dysregulation of one of three cyclin D genes—*CCND1*, *CCND2*, or *CCND3*. *CCND1* and *CCND3* are directly overexpressed as the result of t(11;14) and t(6;14), respectively, while *CCND2* is indirectly upregulated in the t(4;14) and MAF translocation group⁷ (see Table 2). An additional approximately 20% of MM patients harbor *IGH* translocations involving other partner genes.²⁹ The frequency of *IGH* translocations increases from MGUS to MM, suggesting some *IGH* translocations occur as secondary events in patients who present with hyperdiploid MM.²⁹ In the absence of an *IGH* translocation, a diagnosis of κ and λ light chain immunoglobulin gene translocations, which have reported frequencies of up to 20% in MM, should be considered.¹¹ The most common *IGH* translocation partners—4p16 (*FGFR3* and *MMSET*), 11q13 (*CCND1*), 6p21 (*CCND3*), 16q23 (*MAF*), and 20q12 (*MAFB*)—are discussed in more detail in the text that follows, along with associated therapy and outcomes (see Table 3; Table 4). For a comprehensive discussion of the role of immunoglobulin gene rearrangements in antibody diversity and the pathogenesis of MM, see the review by Gonzalez and colleagues.⁹

t(4;14)(p16;q32)

t(4;14)(p16;q32) occurs in approximately 15% of MM patients and results in the direct dysregulation of both the fibroblast growth factor receptor 3 gene (*FGFR3*) and a histone methyltransferase gene referred to as the MM SET domain gene (*MMSET*). *MMSET* is also known as *WHSC1*, or the Wolf-Hirschhorn syndrome candidate gene. Derivative 14 expresses *FGFR3*, an oncogenic tyrosine kinase receptor, and *MMSET* is overexpressed as a result of the der(4) fusion.^{45,49} Approximately 20% of t(4;14)-positive tumors lack *FGFR3* expression, a finding that correlates with loss of der(14).^{50,51} The translocation results in the exchange of two subtelomeric light staining G-bands, so the best methods for detecting this

Table 3 Most common cytogenetic abnormalities in MM			
Abnormality	% Frequency	Other associations	Survival characteristics
Ploidy status			
Hyperdiploidy	~60	48 to 74 chromosomes Gain of the odd chromosomes, esp. 5, 9, & 15; Less likely to show del(13q) or -13	Best OS among the abnormal ploidy subgroups
Non-hyperdiploid	~40	<48 or >74 chromosomes Usually (70%~90%) associated with <i>IGH</i> translocations	Less favorable than hyperdiploid karyotypes
Hypodiploid	~30	≤44 chromosomes Frequent (88%) <i>IGH</i> translocations with ≥50% showing t(4;14)	
Pseudodiploid	~18	45 to 47 chromosomes	
Tetraploid	~1~2	≥75 chromosomes Usually coexist near 2n and 4n clones; Frequent (~90%) <i>IGH</i> translocations	
Primary <i>IGH</i> aberrations			
t(4;14)(p16;q32)	~15	~90% have concurrent del(13q) Indirectly dysregulates <i>CCND2</i>	Poor Short remission (21 to ~30 months) after high dose-chemotherapy and SCT
t(6;14)(p21;q32)	~4	~66% have concurrent del(13q) Directly dysregulates <i>CCND3</i> Indirectly dysregulates <i>CCND2</i> Variant translocation involves <i>IGL/22q11</i>	Similar to MM with t(11;14)
t(11;14)(q13;q32)	~20	~50% have concurrent del(13q) Directly up-regulates <i>CCND1</i> Associated with CD20 expression, lymphoplasmacytic morphology, hyposecretory disease, and λ light chains in serum	Variable (heterogeneous group); poor if presenting with PC leukemia <i>RAS</i> mutations may predict disease progression

(continued on next page)

Table 3 (continued)			
Abnormality	% Frequency	Other associations	Survival characteristics
t(14;16)(q32;q23)	~3	Up-regulates <i>MAF</i> and indirectly up-regulates <i>CCND2</i> Frequent del(13q) and IgA isotype	Suggests high-risk clinical course
t(14;20)(q32;q12)	~1	Up-regulates <i>MAFB</i> Observed in non-hyperdiploid karyotypes (>80%), del(13q) (~75%), and gain of 1q (>50%)	Trend suggests poor risk—similar to t(14;16)
Other IGH translocations	~20	Concurrent del(13q) (~45%)	Little data available—neutral prognosis
Common aberrations—reported as primary or secondary			
Deletion of 1p	~30	Non-specific	Reflects disease progression; determinant of high-risk disease
Gain of 1q21	~35	Non-specific	Reflects disease progression; determinant of high-risk disease
t(8;14)(q24;q32) or MYC variants	2 to ~4	Deregulates <i>MYC</i>	Reflects disease progression
Deletion of 12p	~10	Cryptic deletions at or near <i>CD27</i>	Short EFS and OS
del(13q) or -13*	~45*	Monosomy 13 (~85%) vs del(13q) (15%); reported in ~75% non-hyperdiploid vs ~35% hyperdiploid MM; concurrent with high-risk <i>IGH</i> translocations	Does not appear to affect prognosis in hyperdiploid cases; A marker for clonal expansion in t(4;14) and t(14;16) MM
Deletion of 17p	8~14	Uncommon in MGUS Present in ~10% newly dx cases; MM with ≥60% have ultra-high-risk disease and the poorest prognosis	Rapid disease progression

Table 4 Correlation between MM therapy and cytogenetics		
Abnormality	Relationship with Treatment	References
Deletion 1p	Associated with short remission and survival in patients undergoing high-dose therapy and a single autologous transplant.	Qazilbash et al ⁷⁵
Deletion of 1p21 or 17p	Adversely affects outcome for lenalidomide- plus dexamethasone-treated patients with relapsed or refractory MM. Co-presence of t(4;14) does not appear to be an adverse risk factor for PFS or OS.	Reece et al ¹⁰²
Amplification of 1q	Thalidomide improved 5 year EFS in patient lacking amp(1q21) but not in patients with amp(1q21) and did not improve OS in either group.	Hanamura et al ⁷⁴
del(17p13) or gain of 1q21	Associated with a dismal OS when treated with lenalidomide and dexamethasone.	Klein et al ¹⁰⁴
Absence of metaphase chromosome 13 aberrations and hypodiploidy	Favorable prognostic factor in tandem autologous SCTs.	Shaughnessy et al ¹¹⁷
t(4;14) and low <i>TP53</i> expression	Bortezomib may overcome poor prognosis significance of t(4;14) but cannot overcome the adverse outcome of low <i>TP53</i> expression or the high-risk 17-gene MM model.	Barlogie et al ¹¹⁸
Low <i>TP53</i> gene expression and del(17p13)	Deletion is an independent adverse prognostic marker in newly diagnosed MM treated with autologous SCT.	Xiong et al ³²
<i>TP53</i> deletion	Patients with del17p/ <i>TP53</i> show an extremely poor outcome with VRD (lenalidomide, dexamethasone [RD] and bortezomib) combination.	Dimopoulos ¹⁰³

IGH rearrangement are PC FISH and reverse transcription polymerase chain reaction (RT-PCR) for the hybrid *IGH/MMSET* transcript.^{29,52} The translocation has been reported in both asymptomatic and symptomatic disease.^{45,52} When observed in asymptomatic disease (MGUS and SMM), approximately 25% of cases progress to symptomatic MM in less than a year from diagnosis,⁵³ and associated features include frequent IgA isotype (~50%), concurrent 13q deletion (~90%), high S β ₂M concentration (usually ≥ 2.3 mg/L), up-regulation of *CCND2*, and infrequent presentation with bone lesions.^{7,41,50,52–55} Although the overall initial therapy response rate for this subgroup is good, median progression-free survival is approximately 12 months, and more than one-third of patients experience rapid, aggressive, and drug-resistant relapses.⁵³ High-dose chemotherapy with stem cell transplantation offers better rates of overall response and progression-free survival.^{53,55} In addition, emerging data using proteasome inhibitors (bortezomib) show promise for this poor-risk subgroup (see **Table 4**).^{53,56}

CCND1 and CCND3 Translocations

The t(11;14)(q13;q32) translocation occurs in approximately 20% of MM patients and results in a *CCND1/IGH* rearrangement with concomitant up-regulation of cyclin D1 (*CCND1*). This translocation has been associated with frequent expression of CD20, lymphoplasmacytic morphology, IgM myeloma, hyposecretory disease, λ light-chain serum monoclonal protein of less than 10 g/L, and a lower plasma cell labeling index: it may be seen in hyperdiploid karyotypes.^{29,57–59} The prognostic significance of this translocation in plasma cell dyscrasias is unclear. Although reported in MGUS that has been stable for more than 10 years, patients presenting with t(11;14)-positive plasma cell leukemia show an aggressive course and poor OS.^{7,42,57–59} Other genetic alterations, such as *RAS* mutations, appear to be an important factor associated with disease progression in t(11;14) MM.^{60,61} These data are consistent with the two distinct t(11;14) subgroups emerging from the microarray based studies (see “Molecular Classification of MM” section).^{19,30}

CCND3 translocations are less frequent and are found in only approximately 4% of MM patients. The more common t(6;14)(p21;q32) results in *CCND3/IGH* rearrangement, and the less common variant t(6;22)(p21;q11) results in a *CCND3/IGL* rearrangement.⁶² In general, the prognosis of patients with *CCND1* and *CCND3* translocation positive MM is considered neutral.⁶³

MAF Translocations

MAF translocations t(14;16)(q32;q23) and t(14;20)(q32;q12) occur in approximately 5% of MM patients and confer a poor prognosis,^{64,65} but the Intergroupe Franco-phone du Myeloma has questioned that poor prognostic designation for t(14;16) on the basis of small sample cohorts.⁶⁶ The prognosis appears to be linked to *MAF* and *CCND2* up-regulation, which results in apoptosis resistance.^{67,68} Non-hyperdiploid karyotypes are frequently associated with t(14;16), as are a higher prevalence in IgA myeloma,⁶⁵ a higher frequency of leukemic presentation, and frequent concurrent 13q deletions (78%).⁶⁶ Although the translocation has been detected in MGUS,¹² it confers an aggressive phenotype in MM. The 16q23 breakpoint occurs within the *WWOX* gene, which spans the FRA16D fragile site, raising suspicion that *WWOX* plays a tumor suppressor role in both t(14;16)-positive and del(16q)-positive MM.^{11,69,70} Of interest, a novel *MEK* (mitogen-activated or extracellular signal-regulated protein kinase) inhibitor, U0126, selectively induces apoptosis of *MAF*-expressing myelomas, justifying clinical trials of *MEK* inhibitors for those cases.²⁰

Like t(14;16)(q32;q23), t(14;20)(q32;q12) is strongly associated with del(13q) (76%), non-hyperdiploidy (83%), and gain of 1q (58%).⁶⁵ The translocation has been reported in approximately 1.0% of patients with MM and in 5% with MGUS. Patients with t(14;20)-positive MGUS or SMM may have stable disease for years before progressing.⁶⁵

Secondary Aberrations in MM

Secondary aberrations are in keeping with the multistep progression of MGUS or SMM to MM.^{4,5} These genetic events are not disease defining but may have important implications for prognosis, response to therapy, and potential therapeutic targets. Neither the events nor their sequence within translocation subtypes is well defined. The most common secondary aberrations are described in the following subsections.

Chromosome 1

Chromosome 1 aberrations occur in about one third of patients and are often observed as gain of the long arm with or without deletion of the short arm.^{33,71–79}

Deletions involving chromosome 1 span the entire short arm but most lie within the 1p13p32 region.^{33,75–78,80} The minimal regions of deletion were identified as 1p31p32 in an aCGH study⁸⁰ and as 1p21 in a SNP study.⁷⁸ Deletion of 1p is strongly associated with 1q gains and less strongly with 17p deletions^{76,78} and with an overall poor outcome.^{75,76,78} In a study of 203 MM cases, patients with 1p21 deletions showed shorter progression-free and OS than patients without such deletions. After adjusting for del(13q), del(17p)/*TP53*, t(4;14), and 1q21 gains, multivariate analysis indicated that del(1)(p21) was an independent poor prognostic factor associated with disease progression.⁷⁸ When present with del(17p), del(1)(p21) appears to adversely affect the outcome of patients with relapsed or refractory MM treated with lenalidomide and dexamethasone.⁷⁹

Gains of 1q, observed as tandem duplications, isochromosomes, whole arm translocations, jumping translocations, or segmental duplications of the 1q12q32 region, are thought to result from instability of the 1q pericentromeric heterochromatin.^{71,73,74} Jumping and unbalanced 1q translocations show receptor preference, usually targeting 6q, 8p, 16q, 19q, or 17p.^{71–73} The frequency of 1q gain is approximately 40% in newly diagnosed MM, increasing to approximately 70% in relapsed disease.⁴¹ The minimal critical region of gain within band 1q21³¹ contains many candidate genes, including *PDZK1*, *IL6R*, *BCL9*, *CSK1B*, *PSMD4*, and *ANP32E*.^{31,34,81–83} A second 1q region where gain or amplification is reported to be prognostically significant is 1q23.3.¹⁹

Defined as greater than 2 copies of 1q21 by FISH,⁷⁴ amp(1q21) is associated with disease progression and poor OS.^{33,41,74,79,84} Using aCGH on DNA isolated from PCs from SMM patients, Rosinol and colleagues⁸⁴ showed that the risk of conversion to overt disease was linked to gains of 1q21 and loss of chromosome 13. Similarly, using I-FISH, Hanamura and colleagues⁷⁴ showed that 1q21 gain was associated with a higher risk of transition from SMM to MM. When seen concurrently with t(14;16) and t(4;14), it confers a poorer survival, especially if the 1q copy number increases at relapse. Amp 1q21 has also been associated with adverse prognosis and disease progression in MM patients treated with autologous stem cell transplantation (SCT) and in patients with relapsed or refractory disease treated with bortezomib (see **Table 4**).^{74,78,79,82,85,86} These data support the contention that 1q21 houses genes that trigger disease progression and a more aggressive clinical course.

Chromosome 8

MYC/8q24 rearrangements are thought to be late disease-progression events because of their similar frequency in hyperdiploid and non-hyperdiploid MM⁴⁶; however, a single report suggests a frequency in primary MM as high as 15%.⁸⁷ *MYC*/8q24 duplications and translocations are generally seen in complex karyotypes and are associated with 1q gains.^{46,73,87,88} The 8q region appears to play a complex role in MM. Using high-resolution aCGH, Carrasco and coworkers³¹ defined minimal regions of gains within 8q24.12–q24.13 and 8q24.2–8q24.3, and GEP studies suggest that within the 8q21q24 region, *FABP5*, *YWHAZ*, *EXOSC4*, and *EIFC2* contribute to high-risk disease.³⁴

Chromosome 12

DNA-based microarrays show 12p deletions of variable size in approximately 12% of patients with a minimal deletion region near *CD27* at band 12p13.31.^{19,42} Several studies have linked del(12p) to low expression of *CD27*, leading to high-risk disease and a poor prognosis.^{19,89–91}

Chromosome 13

Chromosome 13 aberrations are subdivided into monosomy 13 (85%) and deletion 13q (15%) with no known difference in prognosis. They are common to hyperdiploid and non-hyperdiploid MM. By I-FISH, the incidence of chromosome 13 abnormalities is 50%, but only 15% of newly diagnosed patients showed chromosome 13 aberrations by CC.^{31,42,52,92,93} The timing of detection and the method of detection is of prognostic significance. Chromosome 13 aberrations are not a high-risk feature when detected only by FISH¹⁸ but are when detected by CC because cells detected by CC are proliferating, and proliferating PCs denote active disease and poor OS.^{28,94} Actually, chromosome 13 aberrations seem to garner prognostic significance based on the company they keep. They are highly correlated with high-risk *IGH* translocations (see **Table 3**),^{42,65,94} and one detailed study reported chromosome 13 aberrations in 57% of patients studied, of whom approximately 40% also showed t(4;14), t(14;16), and/or del(17p).²⁸ In relapsed refractory disease, -13 or del(13q) was observed concurrently with both t(4;14) and del(17p).⁷⁹ Finally, the low prevalence of chromosome 13 aberrations in MGUS and SMM, especially the absence in *IGH* translocation-positive MGUS and SMM, provides compelling evidence that chromosome 13 aberrations are secondary. Their presumed role is related to disease progression perhaps by activation of cyclin D overexpression in *IGH* translocation-specific MM.^{42,94}

In GEP studies involving 13q14, *TRIM13* (also known as *RFP2*) exhibited copy number and expression differences in MM and was linked to poor survival,³¹ although it is not listed in the 17-gene model proposed to define high-risk MM (see “Molecular Classification of MM” section).³⁴ *TRIM14* is a candidate tumor-suppressor gene in B cell chronic lymphocytic leukemia (B-CLL) with significant homology to *BRCA1*.⁹⁵

Chromosome 17

Del(17p) was first reported to confer an adverse prognosis in 1998⁹⁶ and is now considered the most prognostically significant chromosome aberration in MM. The short arm of chromosome 17 houses the tumor suppressor gene *TP53*, a transcriptional regulator that influences cellular response to DNA damage. *TP53* deletions are detected by FISH in approximately 10% (range, 9%–34%) of newly diagnosed MM cases,^{33,96–98} but most occur as secondary events marking disease progression.^{32,98,99} When observed at diagnosis, patients with del(17p) are more likely to have an aggressive clinical course, drug-resistant disease, short survival, plasmacytomas (extramedullary disease), and other high-risk features.^{33,42,79,96,100,101} Most (90%) *TP53* deletions in MM are monoallelic; mutations of the *TP53* gene are rare and exclusively found in relapsed MM samples.³²

In a comprehensive investigation, Xiong and colleagues³² reported that low *TP53* expression correlates highly with *TP53* loss; their results also confirmed that *TP53* loss is a significant and independent adverse prognostic factor in newly diagnosed MM. It is associated with shorter event-free and OS, even in the setting of other high-risk disease features, such as high levels of LDH, a high number of bone lesions, an increased incidence of chromosome 13 deletion, amplification of 1q21, and high-risk translocations. Deletion of 17p predicts short survival regardless of therapeutic approach (see **Table 4**).^{96,97,102–104}

Ultra-High-Risk MM

The definition of ultra-high-risk myeloma varies, but in general it is given as myeloma that is likely to lead to death within 24 months. It is diagnosed in 15% to 20% of MM patients and correlates with International Staging System stage 3, a high plasma cell

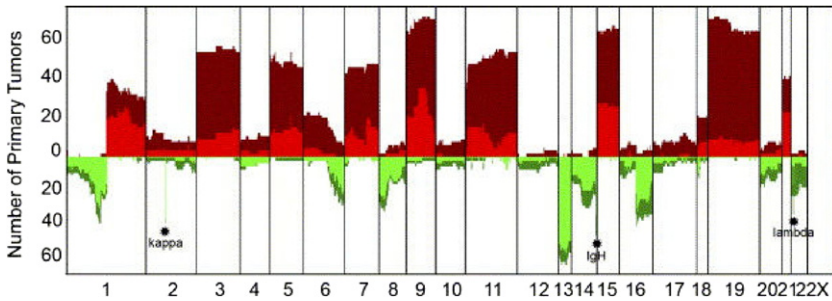


Fig. 5. Composite histogram summarizing the copy number alterations detected by aCGH in samples from 67 newly diagnosed MM patients. The recurring copy number aberrations for each chromosome (x-axis) is plotted against the number of primary tumors (y-axis) showing the aberration. Dark red and green bars indicate the number of samples with gain or loss of chromosome material and bright red or green bars represent the number of samples showing amplification or deletion. Asterisks show focal deletions of the *IGK* (2p12), *IGH* (14q32), and *IGL* (22q11). (Reprinted from Carrasco DR, Tonon G, Huang Y, et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. *Cancer Cell* 2006;9:313–25; with permission.)

proliferative rate, and del(17p).¹⁰¹ A recent report suggests that del(17p) should be considered ultra high risk when seen in 60% or more of bone marrow PCs.⁵⁶ Patients who present with plasma cell leukemia or show poor-risk gene expression profiles (see “Molecular Classification of MM” section) may also be included in this subgroup. Because the t(4;14) subgroup has shown promising responses with proteasome inhibitors, it is considered high risk rather than ultra high risk.^{56,104}

COEXISTENCE OF MYELOID NEOPLASMS AND MM

Therapy-related myelodysplastic syndromes (t-MDS) and acute myeloid leukemia (t-MN) are known complications in MM patients treated with intensive chemotherapy.^{37,105–108} MDS-defined clones may be isolated or mixed among abnormal MM karyotypes. In a single-institution study of 3000 MM patients who received autologous stem cell transplants, approximately 5% showed cytogenetic evidence of MDS.¹⁰⁸ The most common reported t-MN aberrations among MM patients are der(1;7)(q10;p10), del(20q), del(5q), -7/del(7q), +8, del(11q), and del(13q). The presence of del(13q) as a sole aberration should suggest to cytogeneticists the possibility of an underlying t-MN (Fig. 3k,l).^{37,105–108}

MOLECULAR CLASSIFICATION OF MM

High-resolution copy number (aCGH and SNP) microarrays and gene expression profiling are refining the genetic characterization of MM and its prognostic significance.^{19,30–35} I discuss here how comparing molecular genetic data with patient outcomes after current therapeutic approaches will advance the molecular prognostic classification of MM.

Using high-resolution aCGH, Carrasco and colleagues³¹ identified 87 discrete minimal common regions of recurrent copy number alterations (CNAs) in 67 newly diagnosed MM patients (Fig. 5). Of those, 14 CNAs, including TP53 deletions, were found to be associated with poor survival. Using unsupervised clustering, hyperdiploid MM was segregated into two groups, one exhibiting gain of odd-number

chromosomes and another exhibiting gain of 1q and 7, deletion of 13q, and absence of trisomy 11. The study also identified two non-hyperdiploid subtypes, one characterized by high-level amplification of 1q21q22, deletion of 1p, and chromosome 13 aberrations and another characterized by absence of chromosome 1 abnormalities with deletions of chromosomes 8 and 13. In a study using high-density SNP arrays in 192 newly diagnosed MM cases, Avet-Loiseau and colleagues¹⁹ proposed two prognosis subgroups—an adverse prognosis group associated with 1q gain or amplification and deletions involving 1p, 12p, 14q, 16q, or 22q, and a more favorable prognosis group associated with hyperdiploidy (gain of chromosomes 5, 9, 11, 15, or 19). Multivariate analysis with confirmation by an independent series resulted in the identification of del(12)(p13.31) as an independent adverse marker and amp(5q31.1) with low $S\beta_2M$ as a favorable prognostic marker. Generally speaking, these data concur with the known MM cytogenetic data and refine the alterations down to the specific cytogenetic band.

In a combined high-density SNP and GEP investigation of 258 samples from the Medical Research Council Myeloma IX study, Walker and colleagues³³ reported frequent deletions of 1p (30%), 6q (33%), 8p (25%), 12p (15%), 13q (59%), 14q (39%), 16q (35%), 17p (7%), 20 (12%), and 22 (18%) and observed acquired isodisomy or copy number-neutral loss of heterozygosity (CN-LOH) for 1q (8%), 16q (9%), or X (20%). This study and another³¹ confirmed the following: (1) direct associations between *IGH* translocations and del(13q), 1q gain and del(13q), and t(4;14) and del(13q); (2) inverse associations between 1q gain or del(13q) and trisomy 11, and t(4;14) with del(16q); (3) two hyperdiploid subgroups based on the presence of trisomy 11 with or without 1q gain and del(13q); and (4) a clinical disadvantage in hyperdiploid MM with concurrent 1q gain with or without del(13q), when compared with hyperdiploid cases with disomy 1q.

In a large SNP and GEP investigation of the significance of 16q deletions in MM, Jenner and colleagues⁷⁰ delineated two subgroups—those with del(16q) and those with LOH 16q23, which included all t(14;16) cases. The minimal region of deletion in the del(16q) subgroup pinpointed band 16q12, the normal locus for *CYLD*, a negative regulator of the NF- κ B pathway, whereas the LOH 16q23 subgroup showed reduced *WWOX* expression. When either group was observed in combination with del(17p), OS was poor.

Three large GEP studies have refined the classification of MM at the transcriptional level.^{30,34,109} Zhan and colleagues³⁰ identified seven distinct molecular subclasses associated with *CCND* gene expression, the recurrent *IGH* translocations, and hyperdiploidy: HY, MF, MS, CD1, CD2, LB, and PR. The hyperdiploid (HY) group overexpressed many genes that mapped to chromosomes 3, 5, 7, 9, 11, 15, and 19. The MF group comprised cases with ectopic expression of *MAF* genes, which included t(14;16)(q32;q23) and t(14;20)(q32;q11), which led to dysregulation of their common downstream targets. The t(4;14)(p16;q32) cases clustered within the MS group, which highly overexpressed *FGFR3* and *WHSC1/MMSET*. Cases clustered within CD-1 and CD-2 were defined by overexpression of the cyclin D genes. CD-1 included the t(11;14) cases and overexpressed *CCND1*. CD-2 samples included the t(6;14) cases and overexpressed *CCND3*, *PAX5*, *CD20*, and several genes localized to 1p. The LB (low bone disease) group showed low *DKK-1* expression. The PR (proliferation) subgroup was characterized by high expression of numerous cell cycle and proliferation-related genes (eg, *CCNB2*, *CCNB1*, *MCM2*, *CDCA2*, *BUB1*, *CDC2*, and *TYMS*) and cancer/testis antigen genes (eg, *MAGEA6*, *MAGEA3*, *GAGE1*, and *GAGE4*). The PR group showed a strong association with highly complex karyotypes. Approximately 27% of the MM cases studied showed a myeloid/normal plasma cell

gene expression signature (MY), a finding that may reflect a copurification of myeloid and PCs or aberrant expression of myeloid genes in malignant PCs.

Within those seven groups, overexpression of genes localized to 1q was highest in the poor-risk PR and MS groups. HY, CD-1, CD-2, and LB subgroups were associated with superior event-free and OS after high-dose therapy and stem cell transplantation whereas the PR and MF groups were associated with poorer outcomes. Using Kaplan–Meier plots, the CD-1, CD-2, LB, and HY groups were associated with low-risk MM and the MF, MS, and PR cases were associated with high-risk MM, with 48-month estimates of event free survival of 68% versus 31% ($P < .001$) and OS of 79% versus 51% ($P < .001$), respectively. By multivariate analysis, these genetic groups, along with abnormal cytogenetics and elevation of $S\beta_2M$ and LDH, were significant independent predictors of OS. The HOVON group³⁵ independently confirmed the existence of these seven subgroups and added three novel subgroups—the NF- κ B cluster based on the overexpression of positive regulator genes in the NF- κ B pathway, the CTA cluster overexpressing a number of cancer testis antigens and the PRL-3 cluster overexpressing *PTP4A3*, *PTPRZ1*, and *SOC3*. Activation of the NF- κ B pathway either by deletion of NF- κ B inhibitors (such as *TRAF3* or *CYLD*) or by activation of NF- κ B activators (such as *NIK* or *CD40*) has been reported in approximately 20% of MM patients.^{70,110–113}

To improve the molecular classification of high-risk MM, Shaughnessy and colleagues³⁴ developed a 70-gene model based on GEP of which 21 genes (30%) mapped to chromosome 1. Of those, 12 up-regulated genes mapped to 1q and 9 down-regulated genes mapped to 1p. This model carved out approximately 13% of MM patients with shorter complete remissions and event-free and OS. The marked increase in the frequency of high-risk designation from 13% at diagnosis to 76% at relapse provides molecular evidence of disease evolution influencing post-relapse outcome. Multivariate discriminant analysis was used to redesign the 70-gene model to a more manageable 17-gene model for the detection of high-risk disease in a clinical setting. Predictably, 9 (53%) of the 17 genes mapped to chromosome 1, supporting the contention that chromosome 1 aberrations concomitant with altered transcriptional regulation of the affected genes play a role in disease progression.^{33,71,73–78,80}

The Intergroupe Francophone du Myélome also developed an expression-based multivariate model that predicts disease outcome using a 15-gene model.¹⁰⁹ The high-risk group showed chromosome instability and overexpression of the genes controlling essential cell cycle processes whereas the low-risk group overexpressed genes involved in hyperdiploidy and protein biosynthesis. Interestingly, the 15-gene model and the 17-gene model had no genes in common, reflecting differences in the clinical trials, the platforms used, and the redundancy of the biological processes that control cell growth, proliferation, and apoptosis.¹¹⁴

Given the limited microarray data available, international cooperation is needed to validate a unified molecular genetic model that can predict outcome and high-risk MM early in the disease. New therapies that target the pathways unique to high-risk disease, including NF- κ B inhibitors, *FGFR3* inhibitors, and *MEK* inhibitors, are in preclinical trials. The genetic changes identified in MM at all levels of risk and the initial molecular classifications of myeloma are providing the rationale for other molecular targeted clinical trials.²⁰ Moreover, emerging data from microRNA profiling, whole genome and exome sequencing, and studies associating SNPs with drug metabolism and tolerance are beginning to reveal the need to consider the genomes of both the patient and the PC clone when making clinical decisions.^{113,115,116}

SUMMARY AND CONSENSUS RECOMMENDATIONS

Analysis of the cytogenetic alterations that occur in the clonal plasma cells of MM patients, and correlation of those alterations with disease course and treatment response, enables genetic risk stratification. Despite the great progress in therapeutic advances that has been made in last decade, many questions remain as to which patient will benefit from which treatments, and when. Refining risk stratification is the key to approaching such problems. Investigators proposing consensus guidelines for the workup of patients with suspected MM have included guiding principles for risk stratification.

Investigators have also proposed guidelines for standardized criteria for reporting clinical trials and recommendations for allogeneic stem cell transplantation.^{6,16–18} All of these guidelines will pave the way for comparing and consolidating studies on an international level to define a unifying molecular classification system. Prognostic factors such as disease stage and cytogenetics (metaphase and I-FISH) findings are recommended for the initial workup of newly diagnosed patients. Although any cytogenetic abnormality suggests elevated risk, known poor-risk abnormalities include hypodiploidy, monosomy 13, or deletion 13q and del(17p) when detected by conventional cytogenetics and the detection of t(4;14), t(14;16) and del(17p) by FISH (see **Table 2** and **Fig. 3**). Because the genetic abnormalities predictive of poor outcome at diagnosis may be similarly predictive at relapse, patients with standard or low-risk disease should be reevaluated periodically for del(17p) and other cytogenetic features indicative of clonal evolution. FISH testing for chromosome 1 aberrations and microarray-based studies are currently limited to clinical trials. Moreover, emerging data from GEP and DNA-based microarray studies are unraveling the complex molecular network of plasma cell tumorigenesis. Together, these new genetic diagnostic tools in combination with novel therapeutic agents will permit the development of therapies that target the characteristics of individual tumor types and define the patient population likely to respond.

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