Risk Stratification in Multiple Myeloma, Part 1: Characterization of High-Risk Disease

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Abstract: Survival in multiple myeloma (MM) is variable, ranging from several months to more than 15 years. While survival has recently improved with the use of novel therapy, approximately 25% of patients have a median survival of 2 years or less. Accurate identification of high-risk patients, and risk stratification, are crucial in improving outcomes for all patients. In the first part of this two part series, we review the currently identified prognostic factors characterized by disease burden (Durie-Salmon staging system, International Staging System, magnetic resonance imaging, (^{18}F) fluorodeoxyglucose positron emission tomography, presence of extramedullary disease or plasma-cell leukemia), host factors (age, performance status, and renal function), tumor biology (proliferation rate, conventional cytogenetics, interphase fluorescence in situ hybridization, and gene expression profiling), and depth of response to therapy. Efforts have been made to identify ultra–high-risk patients by combining all the identified variables into a unifying comprehensive model. In the second part of this series, we will discuss the significance of these factors in the context of currently available therapies for MM, distinguishing between treatments that only *improve* outcomes of high-risk patients when compared with previous therapies, versus those that overcome high-risk status, thereby reclassifying these patients as standard risk.

Introduction

Multiple myeloma (MM) is an incurable plasma cell neoplasm characterized by variable survival ranging from several months to more than 15 years. With modern therapy, survival in symptomatic MM has increased from a median overall survival (OS) of 3–5 years to a 5-year survival rate of greater than 70% in transplant-eligible patients[1,2] and 50% in elderly transplant-ineligible patients.[3] The improvement in survival, however, is not universal, and approximately 25% of patients have a median survival of 2 years or less.[4,5] Accurate identification of

Table 1. The Elements of Risk Stratification

Disease Burden	Host Factors	Biology of Disease
Durie-Salmon staging system	Age	Lactate dehydrogenase >300 IU/L
International Staging System	Performance status	Plasma cell labeling index ≥1%
MRI (≥7 lesions, diffuse bone marrow involvement) FDG-PET (≥3 lesions, SUV >4.2, presence of extramedullary lesions)	Renal function	Conventional cytogenetics
Extramedullary disease		Interphase FISH - CD138 selection - Immunofluorescence of cytoplasmic Ig FISH
Plasma cell leukemia		Gene expression profiling

FDG-PET=(^{18}F) fluorodeoxyglucose positron emission tomography; FISH= fluorescence in situ hybridization; SUV=standardized uptake value.

these high-risk patients is the first step in improving outcomes not only for these unfortunate individuals, but for all patients. Risk stratification is also essential to accurately compare outcomes from clinical trials. As illustrated by the fact that chromosome 13 deletion is no longer considered a marker of poor prognosis, risk stratification requires consideration of not only the methodology or prognostic factor being used to stratify patients but the treatments used at the time that the prognostic system was validated. Fortunately, there have been developments in both arenas in MM.

Prognostic factors have evolved from tumor burden characterization using the purely clinical Durie-Salmon (DS) staging system and the International Staging System (ISS), to the assessment of molecular heterogeneity by cytogenetics, fluorescence in situ hybridization (FISH), and gene expression profiling (GEP). Concomitantly, the number of US Food and Drug Administration (FDA)-approved classes of drugs in the treatment of MM has increased from just corticosteroids and conventional cytotoxics to proteasome inhibitors and immunomodulatory drugs, with more potent second- and third-generation compounds in these classes being recently approved.

In this article, we first review the currently identified prognostic factors categorized by disease burden, host factors, tumor biology (see Table 1), and depth of response to therapy. In the second part of this series, we discuss the significance of these factors in the context of currently available therapies for MM, distinguishing between treatments that only *improve* the outcomes of high-risk patients when compared with previous therapies, versus those that *overcome* high-risk status, thereby reclassifying these patients as standard risk.[6]

Disease Burden and Stage

Durie-Salmon Staging System and International Staging System

The Durie-Salmon (DS) staging system classifies patients based on tumor burden at diagnosis using standard laboratory measurements of calcium, renal function, hemoglobin, and the number of lytic lesions[7] (see Table 2A). This system is predictive of clinical outcome after standard-dose chemotherapy. One of the limitations of the DS staging system is the interobserver variability in the number of lytic lesions seen on a skeletal survey. Moreover, the DS staging system predates magnetic resonance imaging (MRI) and positron emission tomography-computed tomography (PET-CT), which are much more sensitive in detecting osseous disease. Similarly, the DS staging system does not reflect the availability of the serum free light chain assay that can now identify many oligosecretory and nonsecretory patients.

With the use of high-dose therapy (HDT) and novel agents, the DS staging system—which initially was published in 1976—has become less predictive of outcome. In particular, the rate of renal recovery in patients presenting with renal insufficiency has greatly improved in the era of novel therapies, and these patients can have OS comparable to that of those diagnosed with normal renal function.[8] The use of the DS staging system is increasingly becoming limited to clinical trials, primarily to allow comparisons with historical studies.[9] The more recent International Staging System (ISS), which was published in 2005, examined the outcomes of 10,750 patients in North America, Europe, and Asia treated with standard therapy or autologous stem cell transplantation (ASCT). Serum β_2-microglobulin, serum albumin, platelet count, creatinine, and age emerged as powerful predictors of survival on both univariate and multivariate analysis using a Cox regression model. The following factors did *not* emerge as independent prognostic variables in multivariate analysis: age 65 years or older, serum lactate dehydrogenase (LDH) greater than normal, Eastern Cooperative Oncology Group (ECOG) performance status greater than 3, hemoglobin less than 10 mg/dL, and bone marrow plasma cells of 33% or greater.[10]

The 2 variables that emerged as the most consistent, broadly applicable prognostic factors correlating

Table 2A. Durie-Salmon Staging System With Median Survival by Stage[7]

	Stage I* (Patients with *all* of the following:)	**Stage II***	**Stage III*** (Patients with *one* of the following:)
Hemoglobin, g/dL	>10	Neither stage I nor stage III	<8.5
Number of lytic lesions	1 or less		3 or more
Serum calcium, mg/dL	<12		>12
Monoclonal protein (serum in g/dL, urine light chain in g/24h)	IgG <5 IgA <3 BJP <4 g/24h		IgG >7 IgA >5 BJP >12 g/24h
Median survival, mo	Stage IA, 62 Stage IB, 22	Stage IIA, 58 Stage IIB, 34	Stage IIIA, 45 Stage IIIB, 24

* Patients are further subclassified as A or B based on creatinine <2 or ≥2 mg/dL.
BJP=Bence-Jones protein; IgA=immunoglobulin A; IgG=immunoglobulin G.

Table 2B. International Staging System

	Stage I	**Stage II**	**Stage III**
β_2-microglobulin, mg/L	<3.5	Neither stage I nor stage III	≥5.5
Serum albumin, g/dL	≥3.5		
Median survival, mo	62	44	29

with survival duration were serum albumin and serum β_2-microglobulin. Based only on the serum β_2-microglobulin and albumin levels, the ISS separates patients with MM into stages I, II, and III, which have an associated median OS of 62, 44, and 29 months, respectively (see Table 2B). Additional—although weaker—prognostic factors that emerged on univariate analysis were C-reactive protein, immunoglobulin (Ig) isotype, amount of monoclonal protein, and extent of bone lesions.

Unlike the DS staging system, the ISS should be applied only to patients with symptomatic myeloma. DS stage I, which represents about 20% of patients with MM,[11] includes asymptomatic patients, and therefore introduces lead-time bias in calculations of OS. The ISS, which is superior in terms of objectivity and simplicity, also yields a more even distribution of patients than the DS system. The ISS is not only able to better separate patients in stages I and II, but ISS stage III is associated with a shorter OS than DS stage III (29 vs 38 months, respectively). Thus, the ISS is superior to the DS staging system in identifying a truly high-risk subset of patients. Furthermore, the ISS retains independent prognostic value even in the setting of other accepted prognostic factors such as cytogenetics, FISH, and GEP (discussed further below).

However, the commendable global inclusiveness of the ISS also means that access to bortezomib (Velcade, Millennium) and immunomodulatory agents in 2005 may have been quite variable, not to mention that carfilzomib (Kyprolis, Onyx) and pomalidomide (Pomalyst, Celgene) were just entering clinical trials. Therefore, the 29-month median OS for ISS stage III is likely no longer accurate in 2013. A limitation of both the DS and ISS is the lack of evidence in the relapsed setting.

MRI and FDG-PET

Conventional radiography remains the reference standard for staging patients with MM, as established by the International Myeloma Working Group (IMWG) and International Myeloma Consensus Panels in 2011. However, newer imaging modalities such as magnetic resonance imaging (MRI) and positron emission tomography using (^{18}F) fluorodeoxyglucose (FDG-PET) are receiving more attention.[12,13]

In the largest published study on this topic, of 611 patients, focal lesions were detected in 74% of patients by MRI and in only 56% by skeletal survey. Moreover, only the number of lesions detected on MRI (7 or more) was an independent prognostic factor of survival.[14] Another large single-center study looked at 228 newly diagnosed symptomatic patients, of whom approximately two-thirds were treated with bortezomib or immunomodulatory-based agents. Patients who had a diffuse pattern of bone marrow involvement on MRI had an inferior median survival compared with patients with focal and normal patterns: 40 months versus 60 months versus 102 months, respectively (P<.001).[15] In contrast to these findings, a study from the University of Arkansas of 668 MM patients treated with a tandem ASCT-based protocol found that heterogeneity of diffuse bone marrow signal on short-tau inversion recovery (STIR) sequences did not emerge as an independent adverse feature for OS on multivariate analysis.[14]

In another study by the University of Arkansas group, the prognostic value of FDG-PET and MRI was compared in 239 patients who received a Total Therapy regimen.[16] In multivariate analysis, FDG-PET at

diagnosis was the only imaging modality significantly associated with inferior OS and event-free survival (EFS). The 30-month estimate of OS for was 73% for patients with more than 3 lesions by PET-CT, versus 90% in patients with 3 or fewer lesions. In addition, complete normalization of FDG-PET uptake before autologous transplant correlated with better OS and EFS, with a 30-month OS of 92% versus 71% in those with complete normalization and those without, respectively.

The prognostic value of the number of focal lesions on initial FDG-PET was confirmed in another recent large series of 192 patients with MM who received thalidomide-dexamethasone followed by double autologous transplant.[17] In this study, having at least 3 focal lesions, having a standardized uptake value (SUV) greater than 4.2, and the presence of extramedullary lesions correlated with shorter 4-year OS compared with those with negative FDG-PET.

The most recent consensus recommendations on the management of MM consider FDG-PET to be an emerging modality in the management of MM. Conventional radiography, however, is more cost-effective. It is still regarded as the standard for diagnosis and follow-up, and therefore is included in all clinical trials[13,18]

Extramedullary Disease

Extramedullary disease portends poor prognosis. In a recent study of 1,965 patients, 936 of whom were enrolled in Total Therapy protocols, 5-year OS was 31% for those whose baseline PET scan documented extramedullary disease at diagnosis, compared with 59% for those whose scan did not. Multivariate analysis revealed that extramedullary disease was more prevalent in patients with an elevated centrosome index, a measure of centrosome amplification of genes coding for proteins involved in cell cycle, proliferation, DNA damage, and G(2)/M checkpoints, as determined by GEP. Also, other molecular subtypes that are prone to relapse, including overexpression of the *MAF* gene seen with t(14;16) or t(14;20), were associated with increased expression of proliferative genes.[19] In other studies, del(17p) has been associated with increased extramedullary disease.[20,21]

Plasma Cell Leukemia

Plasma cell leukemia (PCL) is defined in one of 2 ways: (1) if peripheral blood leukocyte count exceeds 10×10^9/L, at least 2×10^9/L are circulating plasma cells, and (2) if peripheral blood leukocyte count is below 10×10^9/L, at least 20% are circulating plasma cells. PCL represents a unique subset of patients with MM. PCL is classified as either primary (pPCL), when this is the initial presenting manifestation of MM, or secondary (sPCL), when it is seen in the context of refractory or relapsing disease.[22] Patients presenting with pPCL have clinical features that include younger age, a higher prevalence of extramedullary involvement with hepatosplenomegaly and lymphadenopathy, more thrombocytopenia, a lower serum M protein level, and renal failure.[23] Among 14 patients with PCL, chromosomal abnormalities were detected in all, with del(13q) in 78% and del(p53) in 43%.[24] Poor survival in pPCL was predicted by a MYC translocation, and both pPCL and sPCL showed ubiquitous inactivation of TP53. Primary PCL has been associated with longer median OS than sPCL (11.1 months vs 1.3 months).[20] In the largest series to date of patients with plasma cell leukemia treated with thalidomide-, lenalidomide- (Revlimid, Celgene), and bortezomib-based regimens (70 patients), the median OS for the whole population was 16 months.[25] Patients younger than 65 years were treated with induction (vincristine, doxorubicin, and dexamethasone or bortezomib and dexamethasone) and a double-intensive melphalan autologous or allogeneic transplant, and had a median OS of 31 months.

Host Factors

Age

Increasing age has been purported to be a poor prognostic factor in MM.[26,27] However, a large study of 550 patients with MM treated with high-dose melphalan and stem cell rescue did not support the finding that age is a negative prognostic factor.[28] With a median follow-up of 18 months, the outcome of 49 patients older than 65 years (median age, 67 years; range, 65–76) was compared with that of 49 younger pair mates (median age, 52; range, 37–64). Groups were matched according to cytogenetics, β_2-microglobulin, c-reactive protein, albumin, and creatinine. All patients received high-dose melphalan-based therapy, with 76% of those in the younger group and 65% of those in the older group completing a second transplant. Treatment-related mortality with the first HDT cycle was 2% in younger patients and 8% in older patients (P=.2). Median event-free survival and OS were 2.8 versus 1.5 years (P=.2) and 4.8 versus 3.3 years (P=.4), respectively. Multivariate analysis showed that age was insignificant for both endpoints. Thus, age was not shown to be a biologically adverse parameter for patients with MM receiving high-dose melphalan with stem cell rescue.[28] Similarly, the ISS study did not find age to be an independent prognostic variable on multivariate analyses.

However, there is an inherent selection bias regarding patients—particularly those who are older—who are considered, referred, and selected for high-dose chemotherapy with autologous stem cell rescue. A selection bias also exists in the ISS, as 70% of the patient data used to develop the ISS came from clinical trials, and only patients

Table 3. Genes That Are Typically Involved in Translocations of Different Partner Chromosomes, Which Can Lead to Activation of Oncogenes

Translocation	Oncogene	Prognosis	Reference
11q13	CCND1	Favorable	Soverini, 2003[136] Stewart, 2005[137]
6p21	CCND3	Uncertain	Shaughnessy, 2001[138]
16q23	MAF	Uncertain	Takimoto, 2008[50] Neben, 2010[70]
20q12	MAFB	Unfavorable	Kuel, 2002[47] Bergsagel, 2005[75]
1q21 gain	CKS1B	Unfavorable	Shaughnessy, 2005[139]
4p16	FGFR3 and MMSET	Unfavorable	Kuel, 2002[47] Bergsagel, 2005[75] Moreau, 2002[93] Fonseca, 2003[140] Avet-Loiseau, 2007[61]

FGFR=fibroblast growth factor receptor; MMSET=multiple myeloma SET domain.

about to start chemotherapy were included.[10] In contrast, in a study evaluating all MM patients in England during 2005–2009, the 5-year relative survival rate ranged from 65.2–70.4% in 15- to 49-year-olds to 13.6–17.4% in 80- to 99-year-olds.[29]

In a recent study of 682 newly diagnosed patients, 23% were 80 years or older. These patients had worse performance status, advanced ISS, and a median OS of 22 months, with 14% dying within 2 months of initial therapy.[26] In a meta-analysis of 1,435 patients 65 years or older enrolled in 4 European phase III trials that included thalidomide and/or bortezomib, the risk for death was increased in patients 75 years or older (HR, 1.44; 95% CI, 1.2–1.72; $P<.001$) in multivariate analysis.[30] Possible explanations for the inferior outcome in this group may be that older patients have more comorbidities, their MM is more aggressive biologically, or they tolerate therapies more poorly. Although this meta-analysis focused on elderly patients in clinical trials, many elderly patients are not even enrolled in such studies.

Performance Status

The evolving field of geriatric oncology distinguishes between age and performance status. For example, in the era of novel agents, 155 patients 80 years or older were evaluated. On multivariate analysis, an ECOG score of 1 or less was independently associated with improved survival ($P=.003$) compared with 2 or greater. Median OS in newly diagnosed patients 80 years or older was 16 months for an ECOG score of 2 or greater, versus 29 months for a score of 1 or less ($P<.001$).[26] ECOG performance status, however, has been criticized for its difficult interobserver reproducibility. Indeed, an ECOG score of greater than 3 was not independently significant on multivariate analysis in the ISS.[10] However, as stated previously, the ISS is skewed towards clinical trial–eligible patients. In a series of 410 people with newly diagnosed MM, multivariate analysis revealed that initial ECOG performance status had a highly significant relationship with survival.[31] Median survival was 43 months for patients with an ECOG score of 0–3, versus 15 months for patients with an ECOG score of 3 ($P<.0001$). In another series of 220 newly diagnosed patients with MM treated with conventional chemotherapy, multivariate regression analysis revealed that patients with a Karnofsky performance status score of less than 70 had a median survival of 20.6 months, whereas those with a score greater than 70 had a median survival of 44.8 months. ($P=.001$).[32]

Renal Function

The presence of renal failure has important prognostic value in MM. In a series of 775 patients published in 2000, renal failure was observed in 29% of patients. During the first year after diagnosis, 58% achieved normalization of plasma creatinine at a median time of 3 months.[27] The need for dialysis translated into poor prognosis, with a median survival of 3.5 months. A 12-month landmark analysis of the same patients showed that reversibility of renal failure was a more important prognostic factor than response to chemotherapy.

In a more recent series of 756 newly diagnosed symptomatic MM patients published in 2007, logistic regression analysis showed that renal failure was independently associated only with ISS and Bence-Jones proteinuria. The median survival of patients with creatinine ≥2 mg/dL at diagnosis was 19.5 months, versus 40.4 months for

patients with creatinine <2 mg/dL (*P*<.001). When multivariate analysis was performed, high creatinine did not emerge as an independent variable. Therefore, when corrected for stage, renal failure had no impact on survival.[8] This is likely attributable to the potency and relatively rapid onset of action of novel agents.

Biology of Disease

Proliferation Rate

Lactate dehydrogenase. Serum lactate dehydrogenase (LDH), which was not available for all patients in the ISS study, was not found to be an independent prognostic variable on multivariate analysis.[10] Other studies have found that LDH does provide a convenient and dependable prognostic indicator in MM. In a series of 1,247 patients with newly diagnosed, symptomatic myeloma, those with LDH greater than 300 IU/L had shorter survival (16 vs 47 months) compared with those with normal LDH levels.[33] The frequencies of anemia and hypercalcemia were significantly higher among those with high LDH than in those with normal LDH. Other studies have confirmed the association of high LDH with worse outcomes.[34,35]

Plasma cell labeling index. The bone marrow plasma cell labeling index (PCLI) is a slide-based measure of the percentage of plasma cells in the S phase of the cell cycle that measures plasma cell proliferative activity. An increased bone marrow PCLI (≥1%) is associated with worse prognosis in MM.[36,37] Data from 57 patients with plateau-phase MM (minimal numbers of residual light-chain restricted monoclonal plasma cell) and a bone marrow PCLI of more than 1% were compared with 105 matched control patients with MM and a bone marrow PCLI of less than 1%.[37] All patients had less than 10% total plasma cells on bone marrow aspirate and biopsy. Patients in the high PCLI group had a median OS of 20 months, compared with 56 months in the low PCLI group (*P*<.001).

In addition, a series of 176 patients treated with standard of care at time of diagnosis—68% of whom had ASCT—were retrospectively analyzed in regard to measurable PCLI at diagnosis and 4 months after initiation of treatment. PCLI response was defined as a reduction of 60% or greater. Patients achieving a greater PCLI response had improved median OS of 54 months, compared with 29 months in nonresponders (*P*=.02). On multivariate analysis, the prognostic value of PCLI response was independent of β_2-microglobulin, elevated creatinine, serum M-spike response, and baseline PCLI.[38] Despite the above studies, PCLI is not widely available and its use is limited. Newer flow cytometric methods are in development as a more sensitive, standardized, and widely available assay to measure plasma cell proliferative rate.

Conventional Cytogenetics

The initiation and progression of MM is influenced by multiple mutations in different pathways and genes, which deregulate the intrinsic biology of the plasma cell. Many of these mutations have been characterized. There are sequential acquisitions of multiple genetic events that lead to disease progression and treatment-resistant disease.[39] The plasma cell acquires additional genetic hits over time, through a branching, nonlinear pathway, leading to intraclonal heterogeneity of the myeloma-propagating cell. It is clear, through recent sequencing data, that there is no single genetic change underlying this process that can be targeted therapeutically. Primary genetic events include IgH translocations and hyperdiploidy, while secondary genetic events include copy number abnormalities (ie, gain 1q), DNA hypomethylation, and acquired mutations.[39] The most clinically relevant of these specific genetic events will be discussed below.

Consensus guidelines from the IMWG support a comprehensive cytogenetic and FISH evaluation in all patients with MM at the time of diagnosis and also at relapse.[40] There is general consensus that if a patient acquires high-risk genetic features at relapse or at progression, that patient is considered to have high-risk disease.[40]

Hypodiploidy by conventional cytogenetics, encompassing pseudodiploid, hypodiploid, and/or near-tetraploid variants, has been shown to carry a worse OS.[41,42] In multivariate analysis including stage, β_2-microglobulin, bone marrow plasmacytosis, treatment type, abnormalities of 13q, hyperdiploidy, and hypodiploidy, a hypodiploid karyotype was the first independent factor for OS (*P*<.001), followed by treatment approach.[41]

People with 48 to 75 chromosomes (ie, hyperdiploidy) show a consistent set of trisomies and fewer structural aberrations. Trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21 are observed in 50–60% of patients.[43-45] The hyperdiploid karyotype is associated with better OS. Of note, primary IgH translocations are found in only 10% of cases of hyperdiploid MM versus 75% of cases of nonhyperdiploid MM.[46,47]

However, cytogenetic analysis, or karyotyping, done in metaphase, can be adversely affected by the low proliferative rate of plasma cells. As a result, cytogenetic analysis detects abnormalities in only 22–40% of cases.[48,49] Metaphase cytogenetics is especially poor at detection of translocations, as evidenced by a study in which only 3 of the 25 translocations found by metaphase FISH (which is not commonly performed now) were detectable by cytogenetics.[49] Certain cytogenetic abnormalities are considered poor risk. These are cytogenetically detected t(4;14), del(17p), and—historically—chromosomal 13 or 13q deletion.[40] Given the increased sensitivity of FISH in detecting translocations, high-risk translocations are discussed later.

Monoallelic loss of chromosome 13 or deletion of its long arm—del(13q)—can be seen in nearly 15% of patients when examined by conventional cytogenetics, and in as many as 50% when FISH is used.[50,51] Chromosome 13 deletion is often a secondary event that is found on relapse, and is associated with shorter survival and low response to treatment with standard or high-dose chemotherapy.[52,53] By FISH, deletion 13 is only a weakly prognostic factor, and is not prognostic at all in some multivariable analyses.[54] This is likely multifactorial, reflecting the high prevalence of the abnormality by FISH, the improved efficacy of novel therapies, and also the high correlation of chromosome 13 deletion with other high-risk groups. For example, MM patients with t(4;14) harbor monosomy 13 in 80% of cases.[55] Deletion of 17p13 leads to loss of the tumor suppressor gene p53.[56,57] Although the prevalence in newly diagnosed MM is only 10%, it is more common in relapsed disease and is considered a secondary genetic event and is often prevalent in PCL.[44,53,58-60] The very poor prognosis of deletion of 17p in patients treated with high-dose conventional as well as novel chemotherapy has been shown by a number of large studies, and has been confirmed on multivariate analysis.[58,61-63]

Finally, in the setting of traditional chemotherapy as well as novel agents, chromosome 1q amplification and deletion of 1p by cytogenetics translates into a decreased OS and progression-free survival (PFS).[64-66] In a study done by Wu and colleagues, 137 patients were treated with bortezomib, doxorubicin, and dexamethasone (VAD) followed by ASCT or ∝-interferon.[64] The prevalence of 1p deletions and 1q gain by cytogenetics was 36% and 40%, respectively. Median OS for patients with deletion 1p, gain 1q, and no chromosome 1 abnormalities was 16, 22, and 62 months, respectively (P<.001). In the setting of novel therapy, abnormalities of chromosome 1 by cytogenetics have shown inferior outcomes. In a study using cytogenetics to evaluate 612 patients treated with Total Therapy 2 with or without thalidomide and Total Therapy 3 with or without bortezomib, PFS (HR, 2.08; P<.001) and OS (HR, 2.65; P<.001) were inferior in the group with gain of chromosome 1q and deletion of chromosome 1p.[65]

Fluorescence in Situ Hybridization

Although karyotyping is widely available, it is laborious and has limited sensitivity. In contrast, the sensitivity of interphase FISH (also known as iFISH) is higher owing to the ability to obtain results even in plasma cells neoplasms that are typically associated with a low proliferative rate.

High-risk abnormalities detected by FISH are t(4;14), t(14;16), and del(17p). Several studies have shown the association of these findings with poor prognosis. In a study of 115 patients, high-risk FISH—defined as the presence of t(4;14), t(14;16), t(14;20) or del(17p)—was seen in 24%, and their median OS was 3.9 years compared with "not reached" for standard-risk patients.[67]

Translocations involving the immunoglobulin heavy chain locus can involve different partner chromosomes and may lead to activation of oncogenes. See Table 3 for examples of translocations and their respective oncogenes. Several large studies of patients treated with conventional therapy, single transplant, or tandem transplant have demonstrated an unfavorable prognosis in patients with t(4;14).[58-61,68] Present in about 15% of patients, this translocation involves the fibroblast growth factor receptor-multiple myeloma SET domain (FGFR-MMSET) gene and is found in monoclonal gammopathy of undetermined significance (MGUS) as well, though less frequently.[58,59,69,]

Initially, several large studies have shown on multivariate analysis that t(4;14) was associated with worse EFS and OS independent of ISS stage.[61,70] This population of patients is more likely to have an immunoglobulin A (IgA) isotype and concomitant cytogenetic abnormalities of chromosome 13 and hypodiploidy.[53,69,71] However, more recent data suggest that the t(4;14) is actually a heterogeneous group and not all patients are necessarily high risk.[72,73] Moreover, based on data with proteasome inhibitors that will be discussed further below, t(4;14) can likely be reclassified as intermediate risk.[74]

The translocation t(14;16) has also been associated with an unfavorable prognosis. It contains the MAF transcription factor family, which is upregulated as a result of this translocation.[75] While the Mayo Clinic, Arkansas Multiple Myeloma Group, and Medical Research Council groups have all shown inferior prognosis, the Intergroupe Francophone du Myélome (IFM) did not confirm this finding in patients treated with tandem ASCT.[2,76-78] Despite a higher incidence of leukemic presentation in the patients with t(14;16), no difference was observed for OS. This inconsistency may be related to small numbers (15 patients in Mayo Clinic study; 30 patients in the IFM study) that limited the statistical power, but also to treatment differences. The Mayo Clinic patients were treated with conventional chemotherapy, whereas 60% of the IFM patients received a double-intensive regimen. Another translocation that upregulates the MAF transcription factor and has also been shown to impart an unfavorable prognosis is t(14;20).[79,80]

There are several reports that 1q amplification and 1p deletion detected by FISH have clinical significance as a poor-risk feature, and are predictive of inferior PFS and OS.[81-84] These studies were conducted prior to the use of novel induction therapies. The last IMWG consensus panel found that there are not yet enough data to suggest routine

use of these markers by FISH to predict prognosis. However, in our study of 30 newly diagnosed patients with gain of chromosome 1 detected by FISH in unselected bone marrow aspirates treated with novel agent triplet induction, response rate and PFS were inferior when compared with historical controls. We found early onset of aggressive extramedullary features, including CNS relapse.[85]

The prognostic significance of chromosome 1 can be demonstrated by the gene expression profiling GEP70 and GEP80 model, as pioneered by the Arkansas Multiple Myeloma Group. GEP70 analysis and numerous other reports have demonstrated that gains of 1q12–1q44 are an independent marker associated with disease progression.[86] In fact, 30% of the genes involved in the GEP70 high-risk score were located on chromosome 1, with most of the downregulated genes located on the short arm and most of the upregulated genes located on the long arm.[72] PSMD4, the gene coding for the 26S proteasome non-ATPase regulatory subunit 4, is one of 3 genes common to and hyperexpressed in both GEP70 and GEP80.[87] PSMD4 resides on chromosome 1 and its expression is highly sensitive to copy number. Both higher PSMD4 expression levels and higher 1q21 copy numbers affect clinical outcomes adversely.

Standard-risk genetic abnormalities portend a better response to therapy with longer duration of response, ultimately translating into a longer OS.[53] Patients who have hyperdiploidy by FISH, or translocations involving the immunoglobulin heavy chain locus on chromosome 14—such as t(11;14) or t(6;14)—appear to have what is called standard risk myeloma, which is observed in 50–60% of patients by FISH.[88,89] Among 108 hyperdiploid MM patients studied by FISH, longer median OS (48 vs 35 months) and increased bone disease compared with 146 nonhyperdiploid patients were observed.[90]

Trisomies have been shown to have a positive prognostic value in patients with high-risk cytogenetic abnormalities. Among patients with high-risk FISH, patients who have at least 1 trisomy have improved OS.[67] The favorable prognosis of hyperdiploidy has been shown both by conventional cytogenetics and in 2 large FISH studies.[51,61] Although our study suggests the favorable prognosis of hyperdiploidy may be overcome by the poor prognosis conferred by concurrent chromosome 1 amplification, larger studies are required.

CD138 selection and immunofluorescence of cytoplasmic immunoglobulin FISH. One of the factors in the outcome of interphase FISH testing, however, may be that the plasma cell burden may play a role in the rate of detection. Typically, for each FISH probe, 200 consecutive nuclei are counted. Based on the 95% confidence intervals derived from healthy individuals, the cutoff is 1.5% for translocations and 1.5–12% for numeric abnormalities.[49] Clearly, the plasma cells in patients with low-burden disease will be diluted in standard interphase FISH analysis by the large number of normal bone marrow cells.

For this reason, the IMWG recommends that all patients, but particularly those with low bone marrow burden of plasma cells, be evaluated for genetic anomalies in either purified plasma cells (eg, by CD138-coated magnetic bead selection of the bone marrow aspirate) or by simultaneous immunofluorescence of cytoplasmic immunoglobulin (cIg FISH).[53,71,91,92] Most of the other published studies with large numbers of MM patients have indeed used one of these plasma cell–enriched FISH techniques.[60,61,76,93]

Neither of these techniques, however, has been widely available. Furthermore, in the majority of the world, bone marrow aspirates need to be sent to referral laboratories. This is a problematic issue, given the known ex vivo fragility of plasma cells, as half of purified MM cells die during the first few days of culture and at 9 days the mortality rate is as high as 95%.[94,95] Indeed, in a series of 983 bone marrow aspirate specimens from newly diagnosed MM patients enrolled in the IFM99 trials, the plasma cell purity was greater than 90% for each patient after overnight shipping, Ficoll-Hypaque gradient density centrifugation to separate out mononuclear cells, and CD138 selection. The median number of purified plasma cells, in contrast, was only 6%. As a result, the number of evaluable specimens for the various probes ranged from 532–746 patients (54–76%).[61]

In our study, which directly compared the reliability, specificity, and sensitivity of conventional FISH with cIg FISH in 75 consecutive bone marrow aspirates, cIg FISH was not of particular value in patients with low-burden disease, as 38% of patients had fewer than 25 cells expressing cIg. However, in the remaining evaluable patients, cIg FISH demonstrated higher sensitivity and specificity. The most striking difference between cIg-FISH and FISH was the vastly higher percentage of plasma cells with any given abnormality when detected by cIg-FISH. For example, in MM with an IgH translocation, the median percent of abnormal cells by cIg FISH versus conventional FISH was 91% versus 9%. For cases with del(17p), the median rates were 54% versus 9%, respectively. Indeed, conventional FISH missed a case of del(17p) and t(4;14). CIg FISH also demonstrated in 1 patient with concurrent MM and myelodysplasia that the del(13q) was in the myeloid cells but not the clonal plasma cells.[96]

In a separate prospective study of 28 patients, FISH with immunomagnetic bead plasma cell enrichment was compared with overnight unstimulated bone marrow culture FISH.[97] In the latter, an abnormality was only detected

Table 4. Incidence of Chromosomal Abnormalities in Patients With Newly Diagnosed Multiple Myeloma

Abnormality	Involved Genes	Mayo Clinic (n=351)* cIg+FISH	IFM (n=983)* CD138 selection+FISH
del(13)	Unknown	54.2%	48%
t(4;14)	FGFR3; IgH	12.7%	14%
t(14;16)	IgH; cMAF	4.6%	Not performed
del(17p13.1)	p53	10.7%	11%

* Total number of patients analyzed; however, each genetic abnormality had different number of analyzable patients.

cIg=cytoplasmic immunoglobulin; FGFR=fibroblast growth factor receptor; FISH=fluorescence in situ hybridization; IFM=Intergroupe Francophone du Myélome.

Table 5. Summary of Risk Stratification by Methodology

Methodology	Standard Risk	Intermediate Risk	High Risk
International Staging System	Stage I: β_2-M <3.5, albumin ≥3.5	Neither stage I nor III	Stage III: β_2-M ≥5.5
Cytogenetics		del(13)* Hypodiploidy	del(13)*
FISH and cIg FISH	Hyperdiploidy† t(11;14) t(6;14)	t(4;14)*	del(17p) t(14;16) t(14;20)
Gene expression profiling			High-risk signature

* High-risk abnormality can be overcome with novel therapy.

† Presence of trisomies improves the poor prognostic outcomes of high-risk disease.

β_2-M=β_2-microglobulin; cIg=cytoplasmic immunoglobulin; FISH=fluorescence in situ hybridization.

in 36% of samples, whereas in the plasma cell–enriched fractions, an abnormality was detected in 89% (*P*<.001). It is important to note that the enrichment occurred within 24 hours of bone marrow aspirate with only 1 failure, which may explain the better yield compared with the IFM study. Given that CD138 selection is better when the percentage of plasma cells is less than 20%, perhaps CD138 selection is superior to cIg FISH, although no study has directly compared the 2 approaches.

The percentage of positive cells—ie, the burden of the molecular abnormality detected—also appears to have prognostic value. In the IFM99 study, loss of 17p was present in a median of 75% of cells (range, 32–94%). Serial analysis (at every 5%) was performed to determine the plasma cell cutoffs with the most prognostic value. For del(17p), EFS was 14.6 months if more than 60% of cells were abnormal versus 34.7 months if fewer than 60% were abnormal.[61] Indeed, when the percentage of plasma cells with del(17p) is low, there appears to be no difference in outcome to those without del(17p).[15]

Using interphase FISH with concurrent immunofluorescent detection of the cytoplasmic–immunoglobulin light chain, the Mayo Clinic group[76] found that 351 patients with MM could be divided into one of 3 risk groups. Patients at poor risk were identified as those with t(4;14), t(14;16), and deletion of 17p13; those at intermediate risk had deletion 13; and those with the best prognosis—the remaining patients—had t(11;14). The associated median OS for each of the 3 groups was 24.7, 42.3, and 51.0 months, respectively.[76] In multivariate analysis, the ability of these mutations to predict inferior PFS and OS has been found to be independent of β_2-microglobulin or ISS.[61,70]

The incidence of high-risk molecular findings appears comparable whether done by cIg FISH or FISH on CD138-selected cells.[61,76] Moreover, the occurrence of these high-risk molecular findings is not infrequent. Three chromosomal abnormalities were associated with adverse prognoses. The genes associated with each abnormality, as well as the incidence of each one, are shown in Table 4.

Extrapolation of these types of results is particularly challenging in community practices, where plasma cell–specific techniques are not routinely employed. This makes the significance unclear of a very low percentage of cells with del(17p), for example.

Gene Expression Profiling

Although FISH better allows us to understand the genetics of MM compared with cytogenetics, it is still limited to a number of mutations. Over the last decade, novel tools have been used to assess molecular heterogeneity of the genes involved in MM and to improve the development

of personalized therapy. On the DNA level, amplifications and deletions can be evaluated through array comparative genomic hybridization, single nucleotide polymorphisms can be analyzed by arrays, and even the myeloma genome has been sequenced.[98-100] On the RNA level, evaluation of alternate splicing as well as the use of quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR) to examine micro-RNA has been used to further our molecular understanding of the disease.[101,102] Here, our discussion will be limited to GEP, which is the technique that has been the best studied in the clinic.

GEP is a tool to assess molecular heterogeneity using a much larger series of genes. The technique involves extracting RNA from a highly purified population of CD138+ myeloma plasma cells retrieved from the bone marrow, and using it to probe a high-density microarray gene chip.[103] Two large studies have investigated gene expression profiling; one from the University of Arkansas group and another from the IFM.[5,104]

Shaughnessy and colleagues, from the University of Arkansas, studied the expression profile of myeloma cells in 532 newly diagnosed myeloma patients treated on 2 protocols incorporating tandem ASCT, and were able to identify 70 genes linked to shorter OS, duration of complete response (CR), and EFS.[5,105] A high-risk score was defined by the ratio of mean expression levels of upregulated to downregulated genes, and was an independent predictor of outcome endpoints in multivariate analysis that included ISS and high-risk translocations. Patients with a high-risk score had a 3-year continuous CR rate of only 20%, whereas those without a high-risk score had a 5-year continuous CR rate of 60%. A 17-gene subset was identified on multivariate discriminate analysis that performed as well as the 70-gene model.

When comparing the Arkansas GEP data with cytogenetics and FISH, 2 findings are particularly noteworthy. First, 30% of the genes involved in the high-risk score mapped to chromosome 1, as previously discussed.[5] Second, not all the patients with del(17p) were high risk by GEP. When examined in the context of GEP-defined risk, TP53 haploinsufficiency did not compromise OS or EFS in the 83% with genomically defined low-risk myeloma. It also did not affect the rate or duration of CR.

Therapy also played a role in outcomes. Compared with Total Therapy 2, Total Therapy 3 incorporates bortezomib into the induction, consolidation and maintenance phases of the therapy.[106,107] Unlike with Total Therapy 2, del(17p) was not an independent deleterious feature in Total Therapy 3, even in the FGFR3+ molecular subgroup. Thus, the prognostic implications of del(17p) may depend on the number of plasma cells with the abnormality, additional FISH abnormalities, therapy, and genome-defined risk status.[108]

Another large study published by Decaux and colleagues from the IFM studied gene expression profiles of myeloma cells in 182 patients at diagnosis.[104] The 15 strongest genes, involved in cell cycle progression and its surveillance, were identified to calculate a risk score that correlated with OS based on high-risk and low-risk groups. The results were confirmed in independent cohort of 853 patients with MM. Overall survival at 3 years in the low-risk and high-risk groups was 91% and 47%, respectively.

Several large clinical trials have shown that before any treatment, a high-risk GEP signature is present in approximately 15% of new cases of MM, and is associated with shorter durations of CR, EFS, and OS.[106] GEP studies of serial samples showed that risk increases over time, with relapsed disease showing dramatic GEP shifts toward a signature of poor outcomes.[109] Moreover, alternative whole-genome techniques are also under investigation, such as comparative genomic hybridization and single nucleotide polymorphism–based mapping arrays.

GEP is heralding an era of personalized medicine in MM. Further work is needed, however, especially to justify the cost in resource-poor settings. For example, the GEP risk stratification profiles in the 2 largest gene expression profiling studies do not share common genes. Data analysis techniques, methods used to assess expression profiling, and validation of genes all require standardization so that gene expression profiling can be applied to a single patient and to the general population.

In summary, MM risk stratification can be performed with a variety of methodologies, as shown in Table 5. Cytogenetic risk stratification definitions are according to the IMWG.

Combining Risk Factors Into Prognostic Models

We have discussed the numerous disease burden, host, and biologic parameters that can identify patients with MM at high risk of progression, each associated with particular strengths and weaknesses. In order to more robustly characterize those patients who have a high risk of early death from progression, several studies have combined the prognostic variables that have most consistently found to be independent predictors in multivariate analysis, namely genetic risk stratification and ISS.[61,70,77]

In the largest such model, the Medical Research Council Myeloma IX trial, of 1,069 patients with newly diagnosed MM requiring treatment, 13.8% were designated as being ultra–high risk by ISS II or III. These patients also had 1 or more adverse genetic lesions, defined as 1q21, del(17p13), t(4;14), t(14;16), and t(14;20). This group had a median OS of only 19.4 months.[77] In another study, of 315 patients, the presence of t(4;14)/del(17p13)

and ISS II/III predicted a 5-year OS of only 41%. The French group also presented preliminary data recently regarding a simplified scoring system; these researchers also found that high LDH was independently prognostic.[110]

In our efforts to identify ultra–high-risk patients, it is tempting to combine all the identified variables into a unifying comprehensive model. However, the simplicity and universal availability of the ISS must not be forgotten.

Interpretation of Response Correlating With Outcomes

Achievement of a Complete Response and Quality of Response

Unlike all the risk factors discussed above that are present at diagnosis, the depth of response to therapy is a risk factor that can only be assessed after patients have been treated. Complete response has long been considered the first step toward curing MM. Prior to the introduction of high-dose therapy plus ASCT, the survival benefit for patients achieving CR was demonstrated in a large study from the Eastern Cooperative Oncology Group on 628 patients.[111]

In the context of trials of HDT with ASCT, achieving CR has also been associated with superior survival.[112-115] In 2 studies looking at single versus tandem ASCT, patients who achieved very good partial response or greater after the first transplant did not benefit from a second transplant in terms of OS or EFS.[116,117]

Perhaps the most convincing evidence for correlation of quality of response with long-term survival after ASCT comes from a meta-analysis of 21 studies including 4,990 patients according to best tumor response reported. The associations between maximal response (CR/near-CR/very good partial response) and OS and between maximal response and PFS were highly statistically significant (P<.0001) for both the prospective and retrospective studies.[118]

The correlation of CR in transplant ineligible patients was examined in a recent meta-analysis of 1,175 newly diagnosed patients enrolled in 3 multicenter trials: GISMM-2001 (melphalan-prednisone vs melphalan, prednisone-thalidomide), HOVON 49 (melphalan-prednisone vs melphalan, prednisone-thalidomide), and the GIMEMA MM0305 (bortezomib-melphalan-prednisone [VMP] vs bortezomib-melphalan-prednisone-thalidomide [VMPT-VT]). Multivariate analysis confirmed that the achievement of complete remission was an independent predictor of longer PFS and OS, regardless of age, ISS stage, and treatment.[119] These findings seem to support the use of novel agents to achieve maximal response, even in elderly patients.

Immunophenotypic Response

The very definition of CR is also evolving. Immunophenotypic response (IR) is measured by multiparameter flow cytometry, and a patient is defined as being in IR when clonal plasma cells are undetectable by multiparameter flow cytometry.[120,121] In a prospective series of 102 patients, disease response was assessed after 6 cycles of induction therapy. Those achieving IR showed significantly better outcomes than those achieving PR in terms of 3-year rates of PFS and a trend toward longer OS. Incorporating the IR status into stringent CR criteria translated into a significantly longer PFS (median not reached vs 35 months) and time to progression (P=.003), but no difference in OS was observed. After multivariate analysis for PFS, only IR status after induction was an independent factor; CR and stringent CR were not significant.[122]

In a more recent series of 241 patients who were enrolled in the Spanish GEM (Genes, Environment & Melanoma) 2000 and GEM 2005 trials and were in CR at day +100 after HDT followed by ASCT, only the presence of high-risk cytogenetics by FISH (HR, 17.3; P=.002) and persistent minimal residual disease by multiparameter flow cytometry (HR, 8.0; P=.005) at day +100 could independently predict unsustained CR.[123] More importantly, for those in IR versus those in less than IR, the time to progression was 71 months versus 47 months and the OS rate at 5 years was 71% versus 60%, respectively (P<.001 for both). Therefore, persistent minimal residual disease and cytogenetic abnormalities can discriminate a subset of CR patients with inferior outcome.

Limitations of Correlating Depth of Response to Outcomes

The above discussion correlating depth of response to outcomes has been challenged because responding patients typically stay on therapy longer, resulting in improved survival outcomes. A more valid approach is to use landmark analysis to compare the outcomes of patients with differing depths of response at a given time. The advantage of this landmark analysis lies in its intent-to-treat design, wherein all possible patients continue to be followed, which is especially powerful when an effective treatment in a cancer such as MM not only provides symptomatic relief, but also arrests the progression of disease.

In fact, the Southwest Oncology Group (SWOG) applied landmark analysis to the outcomes of 1,555 previously untreated MM patients enrolled in 4 SWOG phase II trials. Six-month and 12-month landmark analyses were performed to evaluate outcome in each response.[124,125] In patients without progressive disease, the median survival at 6- and 12-month landmarks in responders was comparable to that of nonresponders at 34 months.[124] In contrast, the median survival at the same 6- and 12-month landmarks for patients who had experienced progressive disease was only 13 and 15 months, respectively. Using the Cox survival model, with response

and progression as time-dependent covariates, survival duration was influenced more by the occurrence of progression than by the occurrence of response. Therefore, the magnitude of response as a single variable did not predict survival. Of note, the immunophenotypic CR discussed above maintained its predictive value even with landmark analysis.[113,123]

It is also unclear if treatment response is truly an independent variable or a surrogate for adverse molecular risk. To answer this question, Haessler and colleagues conducted a multivariate regression analysis concerning 668 newly diagnosed MM patients who were uniformly treated with a tandem autologous transplant regimen, according to the Total Therapy 2 protocol.[126] The prognostic implications of time-dependent onset of CR on OS and EFS were examined in the context of standard prognostic features and GEP. When only standard prognostic features were examined, CR benefited patients regardless of risk factors. However, using GEP risk stratification in 326 patients, a survival benefit of CR only pertained to the small high-risk subgroup of 13% of patients (HR, 0.23; *P*=.001), whereas the remaining majority of patients with low-risk disease had similar survival outcomes whether or not CR was achieved (HR, 0.68; *P*=.128). Therefore, the prognostic value of a CR may not be a truly independent variable, but rather dependent on other risk stratification criteria.[126]

PFS Versus OS as a Clinical Endpoint

Although a detailed discussion of the use of PFS versus OS as an endpoint is beyond the scope of this review, several of the risk factors discussed in this review are associated with inferior PFS but not inferior OS.

When used in an MM trial conducted for the purpose of a new drug application (such as with bortezomib, lenalidomide, and liposomal doxorubicin), PFS is an appropriate indicator of drug activity and is able to determine whether or not a particular treatment modality has efficacy.[127-130] For trials in which the question is when in the treatment course a particular FDA-approved agent is best used, however, improved PFS does not necessarily imply clinical benefit with early use.[131] For example, in trials testing the role of lenalidomide as maintenance therapy for patients with MM after ASCT,[132,133] patients in the control arm are able to take lenalidomide at progression. A well-designed study, therefore, ideally would determine the PFS of patients in the control arm who took the FDA-approved agent under investigation at the time of progression.

Moreover, unlike diseases such as large-cell lymphoma, prolonged PFS in MM is not a surrogate for cure rate or OS.[1,134,135] In MM, almost all active drugs will be used in a patient who relapses repeatedly, so PFS only determines the time at which the next regimen needs to be introduced into the treatment pathway.[131] When no proof exists that OS is prolonged, the use of PFS as a surrogate endpoint may result in prolonged use of new treatments without consideration of risks or cost. As in the case of lenalidomide as maintenance, emerging data suggests that an increased risk of second malignancies exists,[132,133] so caution should be exerted. One randomized trial[133] did show a significant OS benefit in maintenance lenalidomide therapy after ASCT, perhaps outweighing the risk of second secondary malignancy.

Although OS differences are difficult to achieve and require large sample sizes, relying on suboptimal endpoints to change practice may result in increased toxicity and irreversible side effects earlier than is necessary, without true clinical benefit.

Conclusion

In summary, high-risk prognostic factors can be categorized by disease burden, host factors, tumor biology, and depth of response to therapy, as discussed above. In the second part of this series, we will discuss the significance of these factors in the context of currently available therapies for MM, distinguishing between treatments that only *improve* the outcomes of high-risk patients when compared with previous therapies versus those that *overcome* high-risk status, thereby reclassifying these patients as standard risk.

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