Leukemia (2007) 21, 529–534

www.nature.com/leu

ORIGINAL ARTICLE

A practical guide to defining high-risk myeloma for clinical trials, patient counseling and choice of therapy

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Clinical outcomes for multiple myeloma (MM) are highly heterogeneous and it is now clear that pivotal genetic events are the primary harbingers of such variation. These findings have broad implications for counseling, choice of therapy and the design and interpretation of clinical investigation. Indeed, as in acute leukemias and non-hodgkins lymphoma, we believe it is no longer acceptable to consider MM a single disease entity. As such, the accurate diagnosis of MM subtypes and the adoption of common criteria for the identification and stratification of MM patients has become critical. Herein, we provide a consensus high-risk definition and offer practical guidelines for the adoption of routine diagnostic testing. Although acknowledging that more refined classifications will continue to be developed, we propose that the definition of high-risk disease (any of the t(4;14), t(14;16), t(14;20), deletion 17q13, aneuploidy or deletion chromosome 13 by metaphase cytogenetics, or plasma cell labeling index >3.0) be adopted. This classification will identify most of the 25% of MM patients for whom current therapies are inadequate and for whom investigational regimens should be vigorously pursued. Conversely, the 75% of patients remaining have more favorable outcomes using existing – albeit non-curative – therapeutic options.
Leukemia (2007) **21,** 529–534. doi:10.1038/sj.leu.2404516;
published online 18 January 2007
Keywords: myeloma; genetics; prognosis; trials

Introduction

Multiple myeloma (MM) represents the malignant culmination of the clonal expansion of genetically transformed plasma cells. Several pre malignant stages have been described including monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM).[1] The differentiation into stages of progression is most important in distinguishing active MM from the preceding stages, as treatment is usually withheld until actual or impending complications from MM are evident.[2] Clinical trials have failed to illustrate any advantage for the initiation of early treatment, and, given the lack of a curative therapy, the delay of treatment until symptomatic progression remains a reasonable strategy.[3] These are important points as some of the same genetic features of high-risk MM are also found in MGUS and SMM.[4–6] Thus, the use of 'high risk' may rightfully be applied to active and symptomatic disease, but should be used with caution in earlier stages where these findings may potentially predict for early progression but where, in fact, hard data are lacking.

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Received 19 October 2006; accepted 31 October 2006; published online 18 January 2007

Multiple myeloma remains incurable. Nevertheless, with modern treatment approaches up to 70–90% of patients will respond to their initial therapy and as many as 40–50% may achieve a clinical complete remission.[2,7,8] Despite this high frequency of early treatment responses, relapse is ubiquitous even for those achieving a clinical complete response and most patients will ultimately succumb to disease progression or complications. However, the timing of relapse is widely variable with some patients progressing almost immediately and some remaining in remission for many years.[9] Indeed, even with the earliest clinically applied staging systems, the disease is highly heterogenous.[9–11] For example, review of the SEER database from 1998 to 2002 (http://seer.cancer.gov/) demonstrates somewhat surprisingly that 25% of patients lived less than 1 year and 40% of patients lived less than 2 years. Many of these early deaths presumably reflect advanced age, co existent morbidity and treatment-related toxicities as even 'high risk' MM will seldom result in such rapid decline, with the possible exception of primary plasma cell leukemia, which occurs in <5% of patients with a median survival of less than a year.[12,13] As an example of the significant impact of co-morbid conditions, or treatment complications, on mortality, we recently recorded a one-year mortality of 30% in patients presenting in acute renal failure even with aggressive management of renal failure and appropriate anti-MM therapy.[14] Most of these patients are never captured in clinical trials, which, then by design, inflate the expected survival of MM patients overall. Even on clinical trials, patients treated with high-dose corticosteroids have an early treatment-related mortality of at least 5%.

This issue aside there is now an abundance of evidence demonstrating that some of the early deaths reflect aggressive genetic features of the underlying disease.[6,15–28] In contrast, the SEER database also demonstrates that 15% of MM patients were alive 10 years after diagnosis. We will show here that the primary determinant of these heterogeneous patient outcomes are the underlying genetics of the malignant plasma cell and propose that this finding is sufficiently important as to warrant a formal definition of 'high risk disease'. These definitions have broad implications for the counseling of patients, the choice of therapy and the interpretation of clinical investigation. We believe that clinical trials should now adopt these or similar criteria routinely such that outcome results can be interpreted correctly.

Myeloma genetics synopsis

One initial event in the genesis of MM is the translocation of non-random partners that include cyclins D1, D2 and D3, MAF

family members (MafA, MafB and c-Maf) and fibroblast growth factor receptor 3 (FGFR3) to the immunoglobulin heavy chain (IgH) switch regions on 14q32.[29] These recurrent translocations account for approximately 40% of MM cases. The remaining 60% of MM lack translocations but instead are characterized by chromosomal duplication and a resultant increase in chromosome number – predominantly reflected by trisomies of odd numbered chromosomes – (hyperdiploidy).[30–32] Each of these genetic events (IgH translocation and hyperdiploidy) is then unified by the downstream upregulation of either cyclin D1, D2 or D3.[18] This seminal phase of chromosome rearrangement or duplication and disease initiation is followed by further karyotypic instability that often includes deletions/monosomy at chromosome 13q14[32–34] or chromosome 17p13 (p53)[26,28] or amplifications of chromosome 1[35,36] or chromosome 8 (MYC).[37] Somatic activating mutation in genes such as *P53*, *FGFR3*, *NRAS* and *KRAS2* may arise, or secondary translocation may occur by a non-B–cell-mediated mechanisms.[32] A common secondary translocation partner is *MYC*.[37] Recently, we have noted mutation of the non-canonical NFkappaB pathway as a common secondary event in up to 40% of patients (PL Bergsagel, unpublished results).

The net effect of these changes and careful examination of their associated gene expression profiles is that MM can be divided into four major transcriptional subgroups (FGFR3/MMSET, MAF, CYCLIN D, HYPERDIPLOID) and for any of these subgroups a dominant proliferation signature may be superimposed.[18,38,39] In recent analyses the combination of these groupings have variously resulted in seven or eight unique gene expression analyses signatures of newly diagnosed patients with one such signature usually reflecting contaminating non-MM cells.[18,39,40] Thus, six–seven gene clusters have been defined in the various expression profiling studies.[6,18,20,32,38,39,41] In common, however, each of these gene expression-based analyses broadly segregates patients into three highrisk camps – FGFR3/MMSET, MAF and PROLIFERATION.

Genetic features of low-risk disease

t(11;14) and t(6;14) is associated with a neutral prognosis

The t(11;14) and t(6;14) upregulate cyclin D1 and D3, respectively.[18,32,39,42,43] They share a gene expression signature and as such may be considered together, for the purposes of disease biology and clinical outcome.[18] Together they represent approximately 20% of all MM patients.[18,39] The presence of the t(11;14) or t(6;14) is associated with an improved or neutral survival in patients treated with conventional or high-dose chemotherapy and stem cell support.[23,28,39,44–46] This good prognosis extends to the most aggressive regimens being employed in MM therapy.[39] Arguing against a very favorable prognosis, studies of long-term survivors of MM have not revealed any enrichment for t(11;14) patients.[23] There is an association of the t(11;14)(q13;q32) with oligosecretory or light chain only MM, CD20 expression and lymphoplasmacytic morphology.[16,44,45,47]

Hyperdiploidy is likely to be associated with a favorable prognosis

The presence of hyperdiploidy is generally considered favorable and patients with hyperdiploidy can live for extended periods after high-dose melphalan-based therapies.[18,30–32,39,48,49] All studies to date have shown superiority in overall survival and progression-free survival for patients with hyperdiploidy, whether this is detected by flow cytometry determination of DNA content, karyotype analysis[50–52] or gene expression profile.[6,39,53] In a recent publication, we were able to show that this difference in survival was not clearly related to initial responsiveness to treatment (using melphalan based strategies); thus, the difference in outcomes reflects a prolonged remission duration.[54] It should be noted that few studies have yet analyzed the impact of hyperdiploidy by multivariant analysis.

Genetic features of high-risk disease

t(4;14) imparts an unfavorable prognosis

Fifteen percent of patients exhibit the t(4;14).[21,22,24,28,39,44,55,56] At least five large studies in over 1500 patients treated with conventional therapy, single or tandem transplant, with or without thalidomide, have demonstrated a uniformly unfavorable prognosis for this group of patients as measured by gene expression, fluorescent *in situ* hybridization (FISH) or immunohistochemistry.[22,28,44,46,55,56] This patient population is also present in premalignant MGUS, but is more common in smoldering and active MM. The t(4;14) population is enriched in IgA isotype MM and in cohorts of patients with relapsed disease.[28,39,57] A large percentage (50–80%) of these patients will have a coexistent deletion of chromosome 13 and are frequently hypodiploid (loss of chromosomes) on conventional cytogenetics.[28,39]

t(14;16)and t(14;20) impart an unfavorable prognosis

The t(14;16), t(14;20) and rare t(8;20) are detectable in 6–8% of patients.[15,18,39,58–61] The MAF transcription factor family is transcriptionally upregulated as a result of these translocations. As with the cyclins, the MAF translocation share a gene expression signature and as such may be considered together for the purposes of disease biology and clinical outcome.[18,39] In at least two series of patient this patient cohort was associated with a shorter survival among patients treated with conventional or tandem transplant-based chemotherapy.[32,39,62,63] Again this population is enriched for IgA isotype, deletion of chromosome 13 and hypodiploidy.[32,39,62,63]

Secondary events that alter prognosis

Inactivation of p53(17p13) is associated with a poor prognosis

Deletions of 17p13 are detectable in 10% of patients at diagnosis and are associated with a shorter survival after both conventional and high-dose therapy.[25,26,28,46,63,64] This deletion is generally considered to be a progression event and is prevalent in plasma cell leukemia and central nervous system MM.[25,32] Again, a number of series have confirmed the very poor prognosis of MM patients with deletion of p53.[6,26,27,29,63] Interestingly, this deletion is not specifically correlated with other high-risk groups particularly t(4;14) that seems almost mutually exclusive.[46]

Chromosome 13 deletion on metaphase analysis is associated with a poor prognosis

One particularly common genetic marker in MM is deletion of chromosome 13 which is detected in ~50% of patients with abnormal karyotypes so that it was detectable in 10–20% of all patients overall.[32,34,65–68] However, the reported prevalence in MM is 50% when interphase FISH has been applied.[54–56,65–70] Independent of the mode of treatment (standard versus high-dose

chemotherapy) and the mode of detection (karyotype versus FISH), MM cases with deletion 13 are associated with shorter survival and lower response rate to treatment.[32] The net effect of deletion 13 on prognosis is, however, greater when deletion 13 is detected by karyotype than when it is detected by interphase FISH.[19] This is owing to the additive effects of the requirement for a proliferative tumor to produce abnormal metaphases. Indeed, when detected by FISH it is only weakly prognostic and is not prognostic at all in some multivariable analysis.[46] This later finding reflects the high correlation of chromosome 13 deletion and other high-risk groups – with up to 80% of t(4;14) patients also harboring a deletion 13. Nevertheless, when found during metaphase analysis the prognosis is very poor.

Amplification of chromosome 1

Amplification of chromosome 1 in a region that includes the *cks1b* gene is common – being found in around 35% of patients – and is considered a progression event.[32,33,36,38–40,69] CKS1B expression is associated with a proliferation signature in MM patients and by both gene expression profiles and by FISH, it confers a poor prognosis,[36,38–40] however, this is not significant in multivariate analysis when FISH is employed as the diagnostic criterion. Its prognostic impact seems stronger when gene expression data are employed.

The impact of proliferation and tumor burden

By a variety of different methodologies, the presence of high tumor burden or increased proliferation in MM is generally unfavorable.[6,9,10,70–77] Surrogate markers include the serum LDH[71,78] and beta-2-microglobulin,[9,46,72,75,79] whereas more direct assays include the plasma cell labeling index[76,80,81] or gene expression-based signatures.[39] Indeed, a proliferation signature on gene expression profiling identifies 15% of patients with dismal outcome independent of other risk factors including otherwise favorable genetics.[39] Conversely, a low or normal beta-2-microglobulin may identify subsets of FISH-identified high-risk patients (e.g., t(4;14)) in whom prognosis is only marginally worse than for other MM patients.[46] The beta-2-microglobulin is commonly employed as a surrogate for tumor burden,[9,76] and likely serves as an adequate, if imperfect, marker of plasma cell number. Indeed, in the recently described international staging system and in a number of other series, the beta-2-microglobulin retains prognostic significance even in multivariate analysis.[9,76]

The actual beta-2-microglobulin cutoff used to define high risk has variously been reported as 3[46] 4[39] or 5.5 mg/l.[9] In the absence of other guidance, we have selected >5.5 mg/l as the most conservative interpretation of high-risk disease as fully 34% of MM patients may be found within this group.[9] Use of the beta-2-microglobulin alone should then be used cautiously, particularly in the absence of other poor prognostic factors and in the presence of renal failure where poor clearance rather than high tumor burden may be dominant. Although not widely adopted, the plasma cell labeling index[76,80,81] remains another tool for assessing plasma cell turnover and retains prognostic significance in many models.

The value of routine genetic testing

The summary above has led to our recommended classification of high-risk MM (Table 1). Specifically, independent studies involving over 1500 patients[21,28,39,46] have identified a poor prognosis associated with the presence of immunoglobulin heavy chain translocations (t(4;14); t(14;16); t(14;20)), deletion of chromosome 13 by conventional cytogenetics or patients with a clearly proliferative tumor of all genetic stripes. Furthermore, similar large studies have confirmed the favorable prognosis of t(11;14) and t(6;14) or hyperdiploid patients lacking both a proliferation signature and metaphase detected abnormality of chromosome 13 or aneuploidy.

As conventional therapies perform poorly for the 25% of patients (by our definition) with high-risk disease, we now feel confident in endorsing a strong recommendation for the adoption of routine molecular genetic testing in MM patients and a suggested 'basic' panel is proposed (Table 2). It is strongly recommended that all newly diagnosed MM patients be tested at a minimum for the t(4;14), t(14;16) and deletion 17p13 by FISH on clonal plasma cells (CD138 selected, cytoplasmic immunoglobulin restricted or morphologically identifiable) to define high-risk disease. Although the t(14;20) and t(8;14) MAF translocations are also likely poor prognosis markers, they are present in a very small fraction of patients ($\sim$2%) and because some of these patients will be picked up by other poor-risk features such as chromosome 13 deletion or aneuploidy, we did not feel it was cost effective to include these diagnostics here. We have retained the deletion of chromosome 13 by metaphase analysis to identify some of this low frequency MAF 'high risk' group but also to identify those 'good risk' genetic patients who may not fare well owing to acquired secondary genetic events that override the initial genetic insult. Although detection of the presence of the t(11;14) or hyperdiploidy to define low-risk disease is of value, by default in the absence of high–risk genetics, these patients would be considered low-risk and, in the absence of elevated proliferation markers can, for the time being, be lumped together therapeutically. Importantly, the evaluation of low-risk patients should include measurement of the serum beta-2-microglobulin, LDH or PCLI as surrogate markers of tumor burden or proliferation.

Table 1 Risk stratification

High risk (25%) Any of	Good risk[a] (75%) The absence of high risk features and presence of any of:
t(4;14) by FISH	Hyperdiploidy
t(14,16) or t(14;20) by FISH	t(11;14) by FISH
Deletion 17q13 by FISH	t(6;14) by FISH
Deletion 13 or aneuploidy by metaphase analysis	
Plasma cell labeling index >3.0	

Abbreviation: FISH, fluorescent *in situ* hybridization.
[a]Patients should only be considered to be truly low risk if genetic markers are accompanied by a beta-2-microglobulin <5.5 mg/l, LDH <250 and/or a plasma cell labeling index <1.0. Similarly, the presence of a beta-2-microglobulin of <3.5 mg/l may favorably modify the course for otherwise high risk genetic risk patients.

Table 2 Recommended basic test panel

1.	t(4;14)	FISH
2.	t(14;16)	FISH
3.	del 17q13	FISH
4.	del 13	Cytogenetics
5.	Beta-2-microglobulin lactate dehydrogenase	Serum

Abbreviation: FISH, fluorescent *in situ* hybridization.

The arrival of targeted therapies

The detection of either t(4;14), t(14;16), deletion of 17p13 (p53) by FISH, deletion of chromosome 13 or aneuploidy on metaphase analysis or PCLI > 3 will define a population of ~25% MM patients who are in a high-risk prognostic group and who do not generally appear to achieve sufficient benefit from conventional autologous stem cell transplant[21,24,28,39,46] to justify the morbidity and cost of the procedure and who should then arguably be steered towards more investigational therapeutic algorithms soon after diagnosis. In particular, the early introduction of bortezomib seems to overcome at least some of the adverse influence of high-risk genetics.[82,83] Alternatively, the 75% of patients under the age of 70 lacking these poor-risk factors are more likely to benefit from a high-dose melphalan-based approach.[39,46] In transplant ineligible patients, a combination of melphalan, prednisone and thalidomide[84] is recommended for low-risk patients, but the early introduction of bortezomib[82,83] should be strongly considered for high-risk patients. For some patients, the presence of specific genetic markers may lend themselves to clinical trials of targeted therapies, for example FGFR3 kinase inhibitors.[85,86]

Conclusion

We suggest here that a high-risk diagnostic panel should be performed on all newly diagnosed MM patients and the results imparted such that patients may make informed choices regarding therapeutic options. Furthermore, we believe that the collection of this information is imperative in the interpretation of current and future clinical trials and should be immediately adopted in trial design.

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