

1 Diagnosis and Genetic Classification of Multiple Myeloma

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INTRODUCTION

In the past decade we have seen great advances in our understanding of the genetic abnormalities present in multiple myeloma (MM) cells, which is believed to be the culprit in the pathogenesis of this disease.¹ This progress has been, in great part, facilitated by the advent of novel molecular genetic and cytogenetic techniques, as well as the unparalleled power available through the genomic revolution. Furthermore, the continued testing for many of these genetic aberrations in large cohorts of patients has allowed for an increasingly accurate description of oncogenomics using primary patient samples. The translation and testing of this basic knowledge in these patient cohorts has provided clinical relevance that truly spans from the bench to the bedside. While much progress has been made in the understanding of the disease, many questions remain, particularly those capable of addressing progression events from the benign stages and unraveling complex interactions supporting clonal survival and evolution. In this chapter we discuss the knowledge regarding a global overview of genetic aberrations of MM cells, primary genetic lesions, secondary genetic events, and, lastly, their clinical implications.

GLOBAL OVERVIEW OF MM GENETICS

At the top hierarchical level, human MM can be divided into two diseases: hyperdiploid MM (H-MM) and nonhyperdiploid MM (NH-MM).^{2,3} The dichotomy separation of MM into these two entities is appealing from the didactic perspective and is clearly substantiated by an extensive body of literature. The biologic basis for the dichotomy is not clear, and enough exceptions exist so that it only reflects a broad distinction to what appears to be different pathogenetic pathways for clonal plasma cell proliferation.

The first observations of this dichotomy were made by Smadja and colleagues through the careful analysis of a series of MM cases with abnormal metaphases.² They were able to observe that one-half of MM cases appeared to be close to the diploid karyotype, with some versions exhibiting duplications resulting in the 4N versions of the tumor (originally called hypodiploid and hyperdiploid MM). In contrast, the other half of patients have multiple trisomies resulting in a median chromosome count of 53. Subsequently, Debes-Marun and others identified recurrent patterns of chromosome aberrations being present in these two subsets, confirming some homogeneity between all NH-MM leading to the current designation.³ Furthermore, our group was the first to show that this dichotomy is largely dictated by the segregation of the recurrent IgH translocations with NH-MM.⁴ This close association, in addition to the recurrent patterns of association, has led to the currently accepted model that MM can be divided into these two broad categories^{4,5} (Figure 1.1).

It is worth noting at this point that the dichotomy has high relevance for the clinical implications of genetic features in MM; the prognosis overall of H-MM is better than that of NH-MM, although greater precision is required for clinical decision making. This more indolent nature of H-MM, and a presumed greater dependency on the bone marrow microenvironment for growth, has precluded the establishment of human cell lines from H-MM as first proposed by us.⁴ This, of course, becomes highly relevant, given the implications for the applicability of preclinical work done using cell lines mostly representing NH-MM.

The two pathways also have implications with regard to baseline clinicopathologic features. While most patients with H-MM have evident bone lesions, a substantial minority of NH-MM [up to 50% of cases with t(4;14) (p16;q32)] have no lytic bone lesions (Table 1.1). This is likely an explanation of why bone lesions, despite being a

TABLE 1.1. Prevalence of bone disease by genetic category in multiple myeloma				
Series	N	H-MM (%)	NH-MM (%)	P value
Fonseca	365	35/220 (84)	39/145 (73)	0.012
t(4;14)(p16.3;q32)			62	NS
t(14;16)(q32;q23)			54	NS
t(11;14)(q13;q32)			62	NS
Keats	205		19/29 (66)	NS
t(4;14)(p16.3;q32)				
Bergsagel (MRI)	172	62/70 (89)	74/102 (73)	
t(4;14)(p16.3;q32)			16/28 (57)	0.002
t(14;16)(q32;q23)			6/11 (55)	0.04
t(11;14)(q13;q32)			30/32 (94)	0.02
Stewart	72		6/11 (55)	0.72
t(4;14)(p16.3;q32)				

NS, not significant; MRI, magnetic resonance imaging

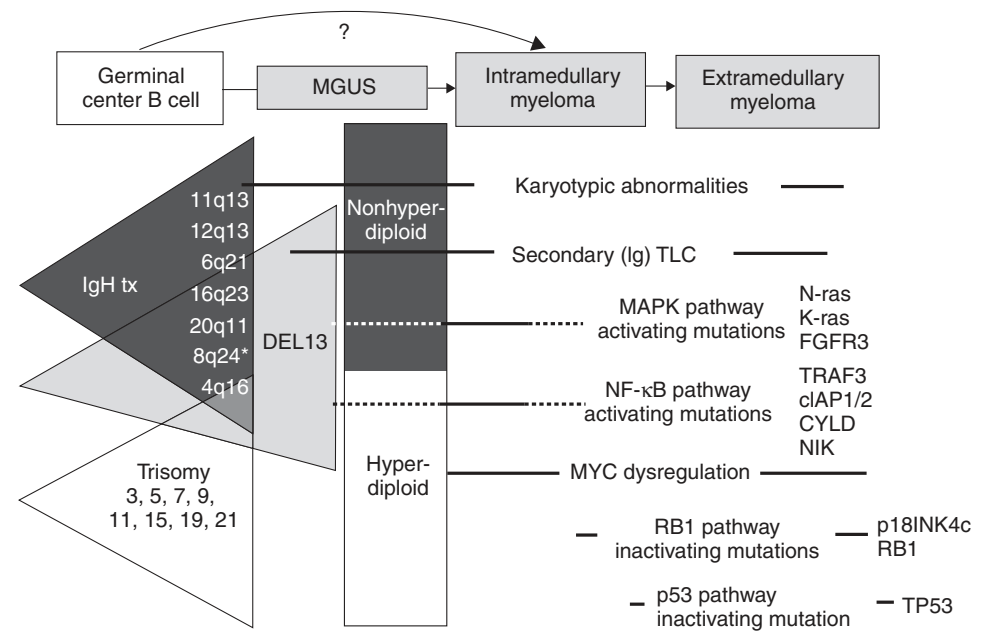


Figure 1.1. Multistep molecular pathogenesis of multiple myeloma (MM). A minimum number of intermediates in the MM pathogenesis pathway are depicted. Horizontal lines indicate the approximate timing of specific oncogenic events, with solid lines indicating the most likely time that these events will occur. MGUS: monoclonal gammopathy of undetermined significance.

sign of advanced disease, have not emerged as an important prognostic factor for MM (i.e., because NH-MM, the more aggressive variant, frequently lacks bone lesions). As one more example, one must remember that plasma cell leukemia, the most aggressive variant of all MM, is rarely

associated with bone lesions.⁶ Hyperdiploid MM is traditionally associated with a more indolent disease, present in higher frequency in elderly male patients, and with a bias for usage of IgG kappa (κ) monoclonal proteins.⁷⁻¹⁰ Again this is likely an explanation of IgA and lambda (λ)

monoclonal proteins as negative prognostic factors (being more common in NH-MM).

The dichotomy between H-MM and NH-MM is evident beginning in the very early stages of the disease.¹¹ Our group demonstrated, using a validated FISH base scoring system, that one-half of the monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM) cases show evidence of H-MM (or H-MGUS), while the recurrent IgH translocations are more common in cases with NH-MM (or NH-MGUS).¹¹ This observation further supports the primordial importance of the biology for the two main subgroups. Furthermore, we have recently established, albeit with a limited number of cases studied, that the overall ploidy category does not change over time.¹² That is, the majority (if not all) of the patients with H-MM will remain H-MM throughout the course of the disease.

PRIMARY GENETIC LESIONS

Immunoglobulin (Ig) translocations

Some original karyotype studies had identified the presence of Ig heavy-chain (IgH) translocations in patients with MM.¹³⁻¹⁵ However, the recurrent partners to these translocations were not readily identified. The only partner that was evident was chromosome 11q13, a translocation easily visible in informative karyotypes.¹⁶ Because the majority of translocation partners had not been identified, no specific clinical or prognostic associations had been associated with these abnormalities. Initially the t(11;14)(q13;q32) was believed to be associated with more aggressive disease, but this was merely due to the fact that patients with karyotype abnormalities, any abnormality, have a more proliferative clone and greater degree of bone marrow involvement.¹⁶ The notion of IgH translocations driving the pathogenesis of MM was appealing since they have been associated with the pathogenesis of many other B-cell neoplasms.¹⁷

In 1995 and 1996, seminal papers were published by Bergsagel, Kuehl, and colleagues, in which they were able to demonstrate that the majority of human MM cell lines harbor IgH translocations, involving the switch regions, presumptively occurring at the time of Ig class switching.^{18,19} This was the first indication that IgH translocations could be more common in MM than was previously suspected. Subsequent work by their team demonstrated that two previously unrecognized translocations, t(4;14)(p16;q32)

and t(14;16)(q32;q23), were each present in 25% of the human MM cell lines.²⁰⁻²² Chesi reported that the genes *FGFR3/MMSET* and *c-MAF* were upregulated respectively in the case of these translocations and by juxtaposition of these genes to the enhancer elements of the IgH genes.²⁰⁻²² Nishida and colleagues used a strategy to detect IgH translocations in primary patient samples (without regard to the chromosome partner) and were able to show that up to 75% of cases had this abnormality (a fraction now known to be higher than the usual ~50%).²³

t(11;14)(q13;q32) and CCND3 translocations

The t(11;14)(q13;q32) is the most common chromosome translocation in MM, present in 15%-18% of cases.^{24,25} This translocation results in the upregulation of cyclin D1 (*CCND1*) with presumptive signaling promoting cell proliferation.²⁶ The translocation also results in upregulation of another gene *MYEOV*, although the consequences of this have not been fully elucidated.²⁷ The translocation is present (as are all other translocations) in the premalignant stages of the disease, with studies reporting a prevalence in MGUS of up to 25% of cases.^{28,29} This indicates that it is not sufficient to promote malignant transformation of plasma cells. However, the translocation is unique among MM genetic changes in that it can be the sole genetic aberration in karyotypes, and it is associated usually with diploidy, likely indicating the need of few genetic changes allowing clonal evolution.^{14,15,30}

The translocation has some unique association with clinicopathologic features such as lymphoplasmacytic morphology, light-chain-only disease, CD20 cell surface expression, and lambda light-chain gene use.^{24,25,31} Overall, patients with t(11;14)(q13;q32) seem to have a more favorable outcome, but most series not showing the magnitude of this trend are such that they reach statistical significance.^{24,25} However, this group is heterogeneous, with some patients having aggressive disease. One study by gene expression profiling showed two subtypes of t(11;14)(q13;q32) possibly dissecting some that would exhibit more aggressive disease.³² Another IgH translocation that involves cyclin D3 (*CCND3*) has been described in 5% of MM cases.³³ While the clinicopathologic implications for this translocation are unknown, it is presumed to be similar to t(11;14)(q13;q32) since at the gene expression level the patterns are nearly identical between the two subgroups.³⁴

t(4;14)(p16;q32)

The t(4;14)(p16;q32) is cytogenetically cryptic such that its detection by karyotype is impossible and it requires FISH, RT-PCR, or gene expression profiling for its detection.^{20,22,30} Chesi and colleagues discovered this translocation in the human MM cell lines.^{20,22} The translocation results in the increased expression of FGFR3 and MMSET.^{20,22} In up to 25% of cases the translocation is unbalanced, always with loss of der14 and consequent loss of expression of FGFR3.³⁵ The translocation is unique in that it is the only one amenable for detection using RT-PCR strategies.^{22,35,36} The orientation of the *MMSET* and *IgG* genes results in a hybrid transcript (IgH-MMSET and MMSET-IgH) that can be detected in the bone marrow and blood of patients with t(4;14)(p16;q32).^{22,35-38} This assay has been used at the research level for diagnostic and disease-monitoring purposes.^{22,35-38}

The t(4;14)(p16;q32) is known to be present in 15% of MM cases and to be associated with more aggressive disease.^{24,35,39} Patients with t(4;14)(p16;q32) have not derived benefit from intensive therapy with high doses of melphalan.^{24,40-42} Patients with t(4;14)(p16;q32) who undergo a single stem cell transplant will usually experience relapse in less than one year and will usually be refractory to alkylators and steroids.^{24,40-42} Only recently, bortezomib has emerged as a possible agent capable of inducing disease control in this subset of the disease.⁴³⁻⁴⁵ The failure with current therapies has led to the development of novel strategies targeting this translocation. A small molecule inhibitor of FGFR3 showed preclinical activity in human MM cell lines with t(4;14)(p16;q32).^{46,47} While preliminary analysis did not show clinically significant activity against the disease, this study exemplifies what in all likelihood will be the future of MM treatments: targeted approaches against the driving genetic lesions.

Patients with t(4;14)(p16;q32) in MM cases are enriched with IgA λ usage and have a high prevalence of chromosome 13 deletions/monosomy.^{24,35,39,48,49} This has been recently implicated as the reason for the negative associations between chromosome 13 deletions and prognosis: that chromosome 13 deletion is a surrogate (if not perfect) marker for t(4;14)(p16;q32). While the t(4;14)(p16;q32) is also observed in the premalignant stages of the disease, it appears to be less common in patients with MGUS and somewhat more enriched in SMM.^{28,29} This would be a possible explanation for the often cited

clinical notion (without much supporting evidence) that MM progressing from SMM is more aggressive [perhaps because of the underlying t(4;14)(p16;q32) biology].

t(14;16)(q32;q23) and other MAF translocations

The last main group of IgH translocations involves the family of *MAF* genes.^{21,32} The most common translocation, also discovered by Chesi, Bergsagel, and colleagues is the t(14;16)(q32;q23).²¹ This translocation is present in 5% of MM cases and is associated with a more aggressive clinical course.^{39,50} Two variants exist that involve MAFa and MAFb but are very rare and only involve less than 2% of cases. It is likely (although currently unknown) that these two translocations will also exhibit the same clinical associations described with t(14;16)(q32;q23).⁵⁰⁻⁵² Like the t(4;14)(p16;q32) patients with MAF, translocations are more frequently associated with IgA λ proteins and have a tendency for enrichment for chromosome 13 deletions.^{39,50}

Other translocations

Several other translocations have been implicated in MM pathogenesis, but none of them are present in a sufficient proportion of cases to know what the clinical implications are. Except for those involving MYC (see below), they will not be discussed further in this chapter.

Trisomies

Aneuploidy is common in MM, but specific patterns are evident.^{7,11} In particular, trisomies are the hallmark of cases of H-MM. While there is evidence of ongoing genomic instability in MM, demonstrated by monosomies and trisomies of all chromosomes, it is only trisomies that are seen in a significant proportion of cases and reproducibly identified by several studies.^{7,11,53} That is, genomic instability alone cannot explain the patterns of predilection for trisomies observed in H-MM.³ Patients with H-MM have trisomies that involve most of the odd-numbered chromosomes, particularly chromosomes 9, 11, and 15. It is notable that trisomy of chromosome 13 is almost never observed (<1% of cases).³ What drives the establishment of trisomies as oncogenic events, and how they contribute to MM pathogenesis, remains unknown. It is presumed now that trisomies contribute in a gene dosage fashion in promoting cell

TABLE 1.2. Translocation-cyclin (TC) classification of myeloma				
Subtype	Prevalence %	Genetics	CCND upregulated	Prognosis
11q13	15	t(11;14)(q13;q32)	D1	Better
4p16	15	t(4;14)(p16;q32)	D2	Poor
MAF	6	t(14;16)(q32;q23)	D2	Poor
D1	42	H-MM	D1	Better
D1 + D2	10	H-MM ?	D1 + D2	Unknown
D2	10	Unknown	D2	Unknown
None	2	Unknown	None	Unknown

survival and proliferation. It appears that several trisomies are required for the establishment of H-MM, so complex interactions must operate in sustaining the clone.⁷

TRANSLOCATION-CYCLIN CLASSIFICATION

A proposal by Kuehl and Bergsagel has been made that almost all MM cases can be said to have a uniform pattern of upregulation of genes associated with the cell cycle (Table 1.2).^{34,51} This classification, the translocation-cyclin (TC) classification, identifies all MM subtypes characterized by a translocation plus evidence of three cyclin D genes upregulation.^{34,51} Patients with H-MM are characterized by increased expression of CCND1, at levels substantially lower than those observed in t(11;14)(q13;q32), but CCND1 is not expressed in normal plasma cells.^{34,51} Patients with the “high-risk” translocations, t(4;14)(p16;q32) and t(14;16)(q32;q23), have downstream upregulation of CCND2. There are two more ill-defined categories of patients with both CCND1 and CCND2 upregulation and patients with none of the aforementioned abnormalities.^{34,51} Provocatively, half of the patients lacking cyclin D expression appear to have no expression of RB (see below), consistent with biallelic deletion, and strongly supporting the role of cyclinD/RB dysregulation in the pathogenesis of MM (Bergsagel, unpublished).

CHROMOSOME 13 DELETION

The first specific genetic lesions associated with prognostic significance in MM were chromosome 13 abnormalities, namely deletions and monosomies. These were identified by the group from the University of Arkansas as associated with shorter survival.^{54,55} While previous work by Dewald and others had identified any metaphase abnormalities

with poor prognosis,¹⁵ chromosome 13 abnormalities were the first specific lesions to be identified.

Chromosome 13 deletions are present in roughly one-half of all MM cases.⁵⁶⁻⁵⁹ It has been subsequently determined that, unlike CLL, MM chromosome 13 abnormalities are mostly monosomies (85% of cases) and less commonly interstitial deletions.^{56,57} Fully elucidating the prognostic contribution of chromosome 13 deletions is complicated

because of its tight association with high-risk genetic lesions such as t(4;14)(p16;q32). It is assumed now, although not conclusively shown, that the negative prognostic implications of chromosome 13 deletions are due to these associations. Chromosome 13 abnormalities are present in MGUS.^{28,60-62}

Many features suggest that indeed chromosome 13 deletions are important in the pathogenesis of MM, including its clonal selection, recurrent nature, high prevalence in some groups such as t(4;14)(p16;q32), and prognostic implications. It is notable that while many chromosomes are trisomic, trisomy of chromosome 13 is exceedingly rare. The area of minimal deletion of 13 is not fully elucidated and is still under investigation.^{56,57,63} Others have suggested *TRIM13* as the putative tumor suppressor gene deleted in cases with chromosome 13 abnormalities.⁶⁴ In our group we have continued to focus on *Rb* as a putative tumor suppressor gene implicated in chromosome 13 deletions. We have found it to be associated with a level of expression that is dose dependent (R Fonseca, unpublished). We have found that introduction of *Rb* slows down cell growth, while reduction in the level of *Rb* expression results in cell growth acceleration. These and other studies continue to explore the role of other genes located in chromosome 13 as pathogenic in MM (R Fonseca, unpublished).

PROGRESSION GENETIC EVENTS

A number of genetic events are believed to play a significant role in the pathogenesis of MM. As has been discussed, the primary genetic events are present in MGUS, many times in cases without progression for more than a decade. As such, they are insufficient to promote full malignant behavior of the clone. Therefore, it is believed

that other factors will facilitate additional cell proliferation or survival.

Most of the factors believed to be secondary will be present across most of the primary genetic subtypes. That is, while, in general, translocations are mutually exclusive, secondary genetic events can be seen in many of the subtypes of the disease, even if clustered or biased for some. It is possible, yet not known, that some genetic events are primarily associated with progression for certain genetic subtypes. For instance, the only factor convincingly believed to be associated with progression from MGUS to MM is *ras* mutational status.⁶⁵⁻⁶⁸ Most recently, we and others have shown that *ras* mutations are more common in patients with t(11;14)(q13;q32) and less so in the other “high-risk” translocations subtypes⁶⁹ (Chng et al., in press, *Leukemia*).

Deletions of 17p13 and p53 inactivation

Deletions of 17p13 have emerged as very important prognostic factors for MM.^{24,39,41,70-73} Multiple series have shown that patients harboring 17p13 deletions, most of them involving *p53*, have shortened survival and more aggressive disease features.^{24,39,41,70-74} Deletions are present in only 10% of cases at the time of diagnosis and are associated with shorter survival, hypercalcemia, extramedullary plasmacytomas, CNS disease, and circulating plasma cells (a feature known to predict for aggressive disease behavior).^{24,39,41,70-74} In the majority of cases of MM with 17p13 deletion there is no concurrent mutation of *p53*, yet patients with and without mutations still exhibit aggressive disease behavior.⁷⁴ We have recently observed that in almost all cases of plasma cell leukemia there is inactivation of *p53*.⁷⁵ We have hypothesized that MM cells are capable of surviving in the absence of bone marrow micro-environment signaling but normally undergo apoptosis upon loss of this signaling in the presence of normal *p53* function. Yet, when cells lose this signaling they become emancipated from the bone marrow signaling, spill into the peripheral blood for circulation, form extramedullary tumoral masses (plasmacytomas), and result in a clinical phenotype of aggressive and nonresponsive disease.

NF- κ B abnormalities

We have recently described a series of genetic aberrations whose common consequence is the upregulation of the

NF- κ B signaling pathway (we think predominantly the noncanonical pathway) (Figure 1.2).⁷⁶ Through a number of mechanisms, positive regulators are hyperactive (by amplification or translocation) and negative regulators are downregulated (by deletion or combination of deletion and mutation). The net result is increased signaling of NF- κ B, documented by increased processing of p100 to p52, and the survival consequences associated with the transcriptional factor effects associated with NF- κ B. While the exact prevalence of these genetic aberrations is not known, we currently believe that up to 25% of MM cases have specific genetic changes leading to the hyperactivation of NF- κ B. As one such example we derived, using gene expression profiling data, an index, we believe, indicates the level of NF- κ B activity.⁷⁶ Using this index we observe that 50% of MM cases exhibit increased NF- κ B expression. In half of these cases we find the specific genetic causes for this activation and in the other half they are yet to be determined.

Further studies of the significance of the abnormality have revealed that in human MM cell lines and primary patient samples with the genetic changes, one consistently observes the downstream consequences predicted by these aberrations. Furthermore, in cases with inactivation of these tumor suppressors (e.g., TRAF3 by biallelic deletion), reintroduction of wild-type TRAF3 abrogates the increased processing of p100 to p52. These genetic changes are all distributed evenly across the major genetic subtypes of the disease, implying their acquisition as progression events in the disease. In summary, the cumulative evidence suggests the important role of constitutive NF- κ B activation in the pathogenesis of plasma cell neoplasms. Clinically this also may be of importance in predicting the likelihood of response to therapy. Certain therapeutics, but particularly the proteasome inhibitors, are believed to induce apoptosis by deregulating the complex interactions associated with NF- κ B survival signaling in MM.⁷⁷ Accordingly our hypothesis has been that cases with constitutive NF- κ B activation should be more dependent on its continued activation for cell survival, and that disrupting this pathway would be more likely to induce cell apoptosis and death, leading to better clinical outcomes. One opportunity to test this hypothesis existed in the context of a pharmacogenomic study associated with a large phase 3 clinical trial of bortezomib in patients with relapsed/refractory MM.⁷⁸ Many patients treated in this clinical trial had samples submitted so that GEP was performed. Since

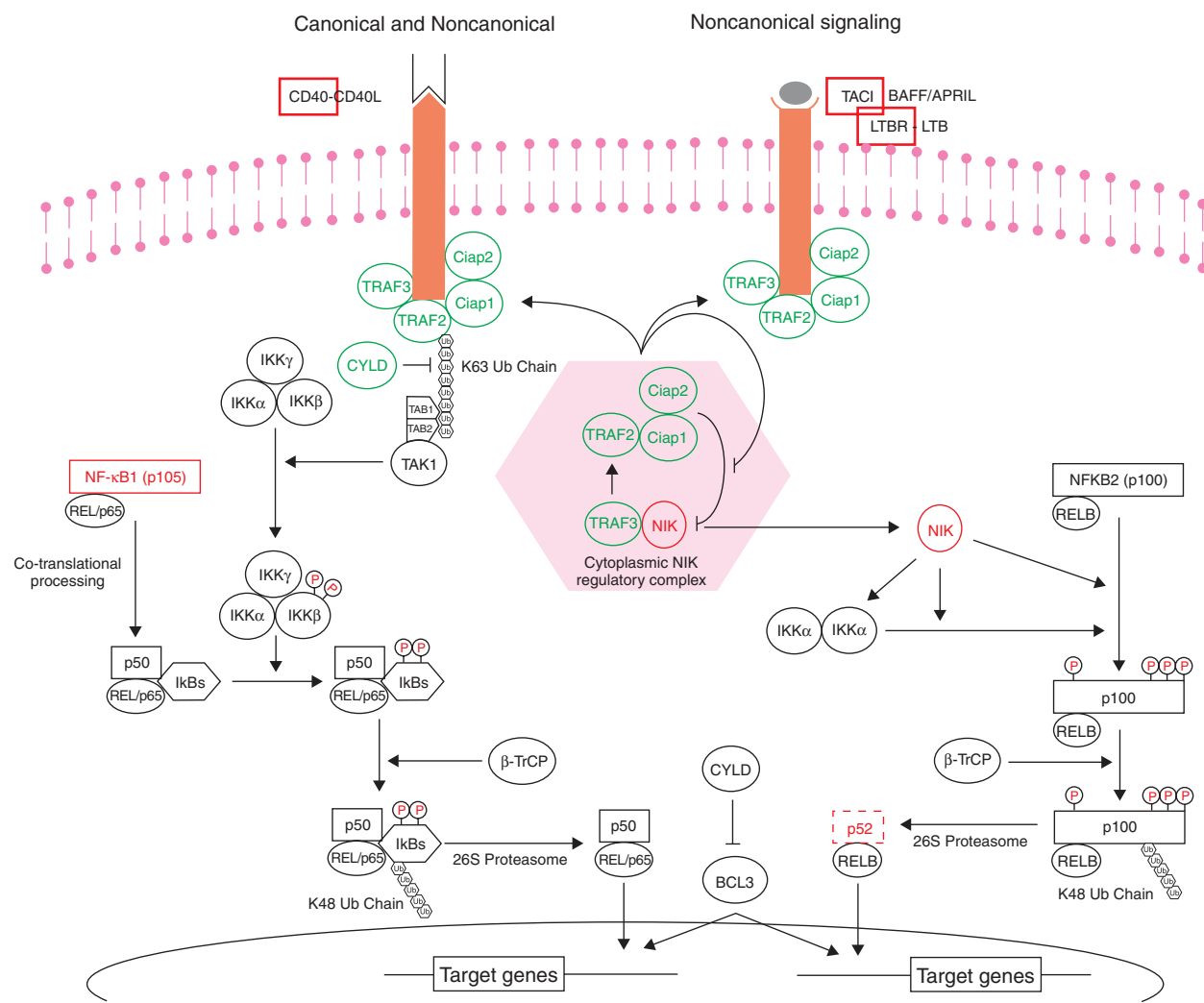


Figure 1.2. Cartoon depicting the genes found by genetic mechanisms contributing to constitutive activation of the NF-κB pathway. Genes depicted in green are those tumor suppressor genes inactivated by deletion and mutation or biallelic deletion. Genes depicted in red are positive regulators of the pathway with increased activity as a consequence of chromosome translocation or amplification. Reproduced with permission from Keats, J et al. *Cancer Cell* 2007; 12(2):131-44.

we did not have DNA available, we could only look at cases with a very low level of TRAF3 as a surrogate for cases with constitutive NF-κB activation.⁷⁶ Using an ROI-derived cut-off value for the level of expression of TRAF3 of 0.6, patients were separated into “low” and “normal TRAF3” levels of expression. Patients who had the normal TRAF3 activity exhibited the usual response rate to bortezomib (~35%), while patients with low levels of TRAF3 expression exhibited a 90% response rate. While these studies can only be considered preliminary, they highlight the real potential

for using genetic categories as predictors of clinical benefit of specific therapies.

One of the major challenges in the clinical applicability of these new findings will be their conversion to practical tools to be used with clinical diagnostic samples. In our original studies mutations were detected in at least 14 NF-κB-associated genes (including true mutations, deletions, translocations, amplifications, etc.).⁷⁶ Accordingly, a comprehensive genomic approach is not feasible in the clinic. Our aforementioned GEP-derived NF-κB index is

not fully validated for clinical use and is awaiting confirmation of larger studies that combine a comprehensive genomic approach of mutation detection alongside gene expression profiling. Furthermore, it is unlikely that GEP will ever be a practical diagnostic tool for MM. It is then that a functional readout of the consequences of increased NF- κ B activity is needed. In all cases where we found mutations we found by Western blot an increased processing of p100 to p52. Performing a Western blot with clinical samples will be complicated, given the requirement of cell selection. Thus, we are left with slide or flow-based, single-cell analysis for evidence of hyperactivation of NF- κ B. We have tested and published the use of immunohistochemistry and immunofluorescence to detect nuclear localization of NF- κ B in MM cells. Potentially, flow-cytometric-based strategies could also be used but can only be done prospectively since they require fresh samples.

MYC abnormalities

Translocations that involve an MYC gene are rare or absent in MGUS but occur in 15% of MM tumors, 44% of advanced tumors, and nearly 90% of HMCL. Mostly, these rearrangements involve c-MYC, but about 2% of primary tumors ectopically express N-MYC (and presumably have N-MYC translocations, as confirmed in some cases), and an L-MYC rearrangement has been identified only in one HMCL. These translocations, often heterogeneous in primary tumors, are usually complex rearrangements or insertions, sometimes involving three different chromosomes.^{32,79-81} An Ig locus is involved in 25% of these translocations: The IgH locus is involved somewhat more than the Ig λ locus, but the Ig κ locus is only rarely involved.⁷⁹ Thus MYC rearrangements are thought to represent a progression event that occurs at a time when MM tumors are becoming less stromal cell dependent and/or more proliferative, whereas biallelic c-MYC expression stimulated by IL-6 and other cytokines occurs at earlier phases of tumorigenesis. Important questions about the role of MYC translocations in MM are raised by two observations. First, Avet-Loiseau and his colleagues found that MYC translocations were rare in primary plasma cell leukemia, a surprising result given the high prevalence in advanced primary tumors and HMCL that are derived from primary and secondary plasma cell leukemia.⁸² Second, a large study by Avet-Loiseau and colleagues showed no effect of

MYC rearrangements on prognosis.⁸³ Unfortunately, they were not able to determine if MYC/Ig rearrangements affect prognosis. In contrast, in an analysis of 596 patients at Arkansas for which GEP data was deposited, patients with tumors that express N-MYC (presumably as a result of a translocation) or express very high levels of c-MYC (normalized value > 4) had a significantly poorer survival than the entire group of patients (WM Kuehl, personal communication). In the C57BL/6 strain of mice, that is predisposed to develop MGUS, late activation of a MYC transgene universally leads to MM, indicating a causative role of MYC dysregulation in the progression of MGUS to MM.⁸⁴

Chromosome 1 abnormalities

A number of labs have determined by a combination of FISH, array comparative genomic hybridization (aCGH), and gene expression profiling (GEP) that there is a gain of sequences – and corresponding increased gene expression – at 1q21 in 30%–40% of tumors. These gains are concentrated substantially in those tumors that have a t(4;14) or t(14;16), or have a high proliferation expression index.⁸⁵⁻⁸⁷ Although not formally proven by examination of paired samples, the gain of chromosome 1q21 sequences may occur de novo in tumors with t(4;14) or t(14;16) translocations, but is associated with tumor progression and an increased proliferative capacity in other tumors. It has been proposed that the increased proliferation in tumors with gain of 1q21 sequences is due to the increased expression of *CKS1B* as a result of an increased copy number.⁸⁸ One might expect to find other mechanisms, such as localized amplification or a translocation, if increased *CKS1B* expression is a cause of increased proliferation, but there is no evidence for other mechanism to increase *CKS1B* expression. Furthermore, *CKS1B* expression correlates closely with the expression of a number of proliferation genes in a wide variety of tumors where it appears to be a consequence rather than a cause of the proliferation. So, it seems prudent to remain skeptical that *CKS1B* is the gene targeted by gain of 1q21 sequences.

A large study from UAMS established that 1q21 amplification detected by FISH is a significant and independent poor prognostic factor.⁸⁷ However, another study from the Mayo Clinic shows that while significantly associated with poor prognosis on univariate analysis, 1q21 gain was not an independent prognostic factor on Cox proportional hazard analysis.⁸⁶ The discrepancies in the results from UAMS

and the Mayo Clinic in terms of the independent prognostic impact of 1q21 gain by FISH may be related to differences in the factors included in the Cox proportional hazard analysis. In the Mayo Clinic analysis, the prognostic impact of 1q21 gain was no longer significant when the plasma cell labeling index and t(4;14) were included in the modeling, suggesting that much of the prognostic impact of 1q21 gain on univariate analysis is mediated through its close association with poor risk genetics and proliferative disease.⁸⁶

As mentioned earlier, *CKS1B* has been implicated as the candidate gene on 1q21 mediating biological and prognostic impact. However, when the relative prognostic strength of 1q21 copy gain and increase *CKS1B* expression is analyzed in a multivariate model, 1q21 copy gain is the more significant prognostic factor.⁸⁶ Therefore, the overall evidence that a critical gene located on 1q21 may be causatively involved in mediating progression and prognosis is weak. Instead, it appears more likely that chromosome 1q amplification is a marker of more clonally advanced and genomically unstable tumors that are more likely to progress. The gains on 1q are frequently associated with deletions of 1p, which has also been associated with a poor prognosis.^{89,90}

p16 and p18 abnormalities

It is conceivable and logical that additional hits favoring cell cycle progression could be implicated in the pathogenesis of MM. Inactivation of *p16* via methylation has been observed in up to 50% of MM cases and has been reported as associated in possible familial associations of MM.⁹¹⁻⁹⁵ Deletions of *p16* are uncommon.⁹³ We have recently found that the presence of *p16* methylation confers no significant prognostic association, and thus its role in the pathogenesis of the disease remains unknown.⁹³ Two recent studies showed that most MM tumors express little or no *p16*, regardless of whether the gene is methylated.^{95,96} This suggests that low expression mostly is not due to methylation, which may be an epi-phenomenon. Despite one example of an individual with a germline mutation and loss of the normal *p16* allele in MM tumor cells,⁹⁷ it remains unclear if inactivation of *p16* is a critical and presumably early event in the pathogenesis of MM.

In contrast, it seems apparent that inactivation of *p18INK4C*, a critical gene for normal plasma cell development, is likely to contribute to increased proliferation. Biallelic deletions of *p18* have been observed in 10% of MM

cases and are also believed to be involved in the pathogenesis of the disease.⁹⁸⁻¹⁰¹ Forced expression of *p18INK4C* by retroviral infection of HMCL that express little or no endogenous *p18* substantially inhibits proliferation. Paradoxically, about 60% of HMCL and 60% of the more proliferative MM tumors have increased expression of *p18* compared to normal plasma cells. There is evidence that the E2F transcription factor, which is upregulated in association with increased proliferation, increases the expression of *p18*, presumably as a feedback mechanism. Apart from the lack of a functional RB1 protein in approximately 10% of HMCL, the mechanism(s) by which most HMCL and proliferative tumors become insensitive to increased *p18* levels is not yet understood.

High-risk gene expression profiling

The advent of gene expression profiling has allowed further refinements to our ability to prognosticate patients. In particular, the group from the University of Arkansas has been able to identify a genetic signature indicative of “high-risk” disease and present in 15% of MM cases (Figure 1.3).⁵⁰ These individuals were characterized by evidence of high proliferative disease and included patients with many of the major genetic categories.⁵⁰ The signature initially was derived from a composite analysis of 70 genes but could subsequently be reduced to 17 genes. The conversion of this signature to practical clinical tools could allow identification of cases with more aggressive disease. The correlations of the signature with other validated clinical methods of proliferations assessment such as flow-based S-phase and the plasma cell labeling index would be of great interest. In any case this signature has been internally validated by the same group and others,¹⁰² and identifies cases with more aggressive disease. Other means to identify genetic signatures of aggressive disease such as a centrosome index have also been postulated.¹⁰³

CLINICAL IMPLICATIONS OF GENETIC SUBTYPES

High-risk disease patients have had shorter survival whether treated by conventional forms of alkylator therapy or with high-dose therapy with stem cell support. These observations lead to the recommendations by our group

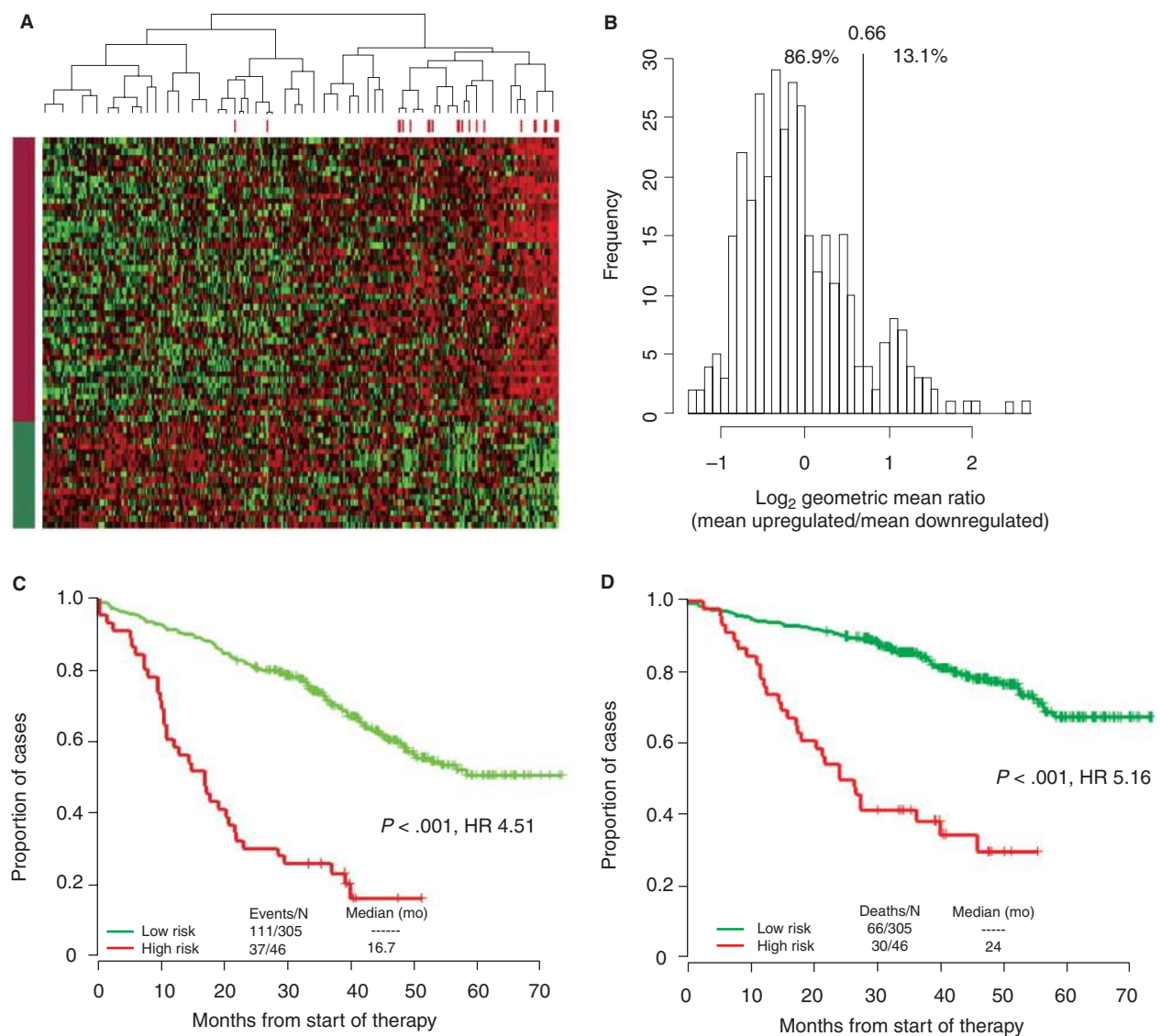


Figure 1.3. GEP determination of a high-risk signature. The red curve depicts the survival of these patients being substantially shorter than that of patients without the signatures. Reproduced with permission from Shaughnessy, JD et al. *Blood* 2007; 109(6):2276-84.

that patients with high-risk MM derive less benefit from the latter intervention and should be considered for alternative management strategies.¹⁰⁴

**Consensus clinical implications:
high-risk disease**

There is a need to develop clinically applicable tests to identify patients with more aggressive disease.¹⁰⁴ To this effect our

group has proposed the establishment of a molecular cytogenetic classification based high-risk disease (Figure 1.4).¹⁰⁴ This group is composed, at the genetic level, of patients with t(4;14)(p16;q32), t(14;16)(q32;q23) or 17p13 deletions. The group is composed of nearly 25%-30% of MM cases and, traditionally, would have identified groups of patients with a median survival of 2 years or less. We also recognize other means by which a patient can, and should, be identified as being in the high-risk genetic category and include