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Genetic events in the pathogenesis of multiple myeloma

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Abstract

The genetics of myeloma has been increasingly elucidated in recent years. Recurrent genetic events, and also biologically distinct and clinically relevant genetic subtypes of myeloma have been defined. This has facilitated our understanding of the molecular pathogenesis of the disease. In addition, some genetic abnormalities have proved to be highly reproducible prognostic factors. With the expanding therapeutic armamentarium, it is time to include genetic assessment as part of clinical evaluation of myeloma patients to guide management. In this review we examine the role of various genetic abnormalities in the molecular pathogenesis of myeloma, and the use of such abnormalities in disease classification, prognosis and clinical management.

Keywords

genetics; gene expression profiling; molecular pathogenesis; prognosis; IgH translocations; hyperdiploid

Multiple myeloma (MM) is an incurable plasma-cell (PC) malignancy. In 2007, it is estimated that 19,900 new cases will be diagnosed, with 10,790 patients succumbing to the disease.¹ In many instances it is preceded by a pre-malignant tumor called monoclonal gammopathy of undetermined significance (MGUS), which is the most common lymphoid tumor in humans, occurring in approximately 3% of individuals over the age of 50.² Both MM and non-IgM MGUS show a similar marked increased prevalence with age. Significantly, the prevalence of both MM and non-IgM MGUS is about twofold higher in African Americans compared to Caucasians, whereas it appears that the frequency of progression from non-IgM MGUS to MM is similar in these two populations.³ Despite some evidence for familial clustering of MM and non-IgM MGUS, the effects of genetic background and environment remain to be clarified.⁴

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Genetic events in MM pathogenesis

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MM IS A PLASMA-BLAST/PLASMA-CELL TUMOR OF POST-GERMINAL-CENTER B CELLS

Post-GC B cells that have undergone productive somatic hypermutation, antigen selection, and IgH switching can generate plasmablasts (PBs), which typically migrate to the bone marrow (BM) microenvironment that enables differentiation into long-lived plasma cells (PCs).⁵ Importantly, non-IgM MGUS and MM are monoclonal tumors that are phenotypically similar to PBs/long-lived PCs, including a strong dependence on the BM microenvironment for survival and growth.⁶ In contrast to normal long-lived PCs, non-IgM MGUS and MM tumors retain some potential for an extremely low rate of proliferation, usually with no more than a few per cent of cycling cells, until advanced stages of MM.⁷

STAGES OF MM

It is thought that a PC tumor must include about 10^9 cells to produce enough monoclonal Ig (M-Ig) or monoclonal IgL (M-IgL) to be detected by serum and/or urine electrophoresis.⁸ However, the recent development of a serum free IgL assay has significantly reduced the number of PC tumor cells that can be detected.⁹ This includes an increased ability to detect low levels of M-Ig (or M-IgL in the approximately 15% of MGUS and MM tumors that express only IgL).¹⁰ For MGUS, serum M-Ig usually is 0.5-3 g/dL, with the tumor cells comprising no more than 10% of the mononuclear cells in the BM.¹¹ Depending on the level of M-Ig, presumably a surrogate for tumor mass, MGUS can progress sporadically to MM expressing the same M-Ig with a probability of about 0.6-3% per year.¹² For a given M-Ig level, an increased level of serum free IgL also is associated with an increased probability of progression to MM.¹³ Unfortunately, there are no unequivocal genetic or phenotypic markers, despite a recent report of extensive gene expression profiling, that can distinguish MGUS from MM tumor cells.¹⁴ Moreover, it still is not known to what extent intrinsic genetic or epigenetic changes in the MGUS tumor cell versus extrinsic changes in non-tumor cells affect progression. Therefore, it still is not possible to predict if and when this progression will occur.

Smoldering MM (SMM) - which has a stable BM tumor content of 10-30% but no osteolytic lesions, anemia, or other secondary manifestations of malignant MM - has a high probability of sporadic progression to frankly malignant MM. Extramedullary MM is a more aggressive tumor that is often called secondary or primary plasma-cell leukemia (PCL), depending on whether or not preceding intramedullary MM has been recognized. Human MM cell lines (HMCLs), which are presumed to include most oncogenic events involved in tumor initiation and progression of the corresponding tumor, have been generated mainly from a subset of extramedullary MM tumors.⁶

IG TRANSLOCATIONS ARE PRESENT IN A MAJORITY OF MM TUMORS

Like other post-GC B-cell tumors, translocations involving the IgH locus (14q32) or one of the IgL loci (κ , 2p12 or λ , 22q11) are common.¹⁵ Mostly they are mediated by errors in one of the three B-cell-specific DNA modification mechanisms: VDJ recombination, IgH switch recombination, or somatic hypermutation. With rare exceptions, these translocations result in dysregulated or increased expression of an oncogene that is positioned near one or more of the strong Ig enhancers on the derivative 14 translocated chromosome.^{16,17} However, translocations involving an IgH switch region uniquely dissociate the intronic from one or both 3' IgH enhancers, so that an oncogene might be juxtaposed to an IgH enhancer on either or both of the derivative chromosomes, as first demonstrated for FGFR3 on der(14) and MMSET on der(4) in MM.⁶ These IgH translocations are efficiently detected by fluorescence in-situ hybridization (FISH) analyses. Large studies from several different groups show that the prevalence of IgH translocations increase with disease stage: about 50% in MGUS or SMM,

55-70% for intramedullary MM, 85% in PCL, and >90% in HMCLs.^{6,18-21} Limited studies indicate that Igλ translocations are present in about 10% of MGUS/SMM tumors and about 15-20% of intramedullary MM tumors and HMCLs. Translocations involving an Igλ locus are rare, occurring in only 1-2% of MM tumors and HMCLs.^{6,18}

SEVEN RECURRENT IGH TRANSLOCATIONS APPEAR TO REPRESENT PRIMARY ONCOGENIC EVENTS

Recently, IgH translocations involving cyclin D2 and MAFA have been reported.²² Thus, there are now seven recurrent chromosomal partners and oncogenes that are involved in IgH translocations in approximately 40% of MM tumors. There are three recurrent IgH translocation groups:^{6,23-28}

1. CYCLIN D: 11q13 (Cyclin D1), 15%; 12p13 (Cyclin D2), <1%; 6p21 (Cyclin D3), 2%;
2. MAF: 16q23 (c-MAF), 5%; 21q12 (MAFB), 2%; 8q24.3 (MAFA), <1%; and
3. MMSET/FGFR3: 4p16 (MMSET and usually FGFR3), 15%.

The recurrent translocation breakpoints usually occur within or near switch regions, but sometimes occur within or near VDJ sequences, suggesting that these translocations are mediated by errors in IgH switch recombination or somatic hypermutation. Since there is no evidence that IgH switch recombination or somatic hypermutation mechanisms are active in normal PC or PC tumors, it is presumed that these translocations usually represent primary - perhaps initiating - oncogenic events as normal B cells pass through germinal centers. With the exception of FGFR3 (especially with an activating mutation)²⁹ and possibly c-MAF,³⁰ the consequences of these translocations have not been adequately confirmed as essential for maintenance of the tumor and/or as therapeutic targets.

CYCLIN D TRANSLOCATION GROUP

The t(11;14) translocation in MM is unusual in that the translocation breakpoints involve VDJ and switch regions with a similar frequency. By contrast, t(4;14) and t(14;16) breakpoints are always and mostly, respectively, located within or near IgH switch regions.^{16,31} The prevalence of t(11;14) translocations is approximately 15% in both MGUS^{18,32} and MM,^{21,33} but the prevalence is >40% in primary amyloidosis (AL),³⁴ despite the fact that AL is thought to be MGUS with a minimal tumor mass and a monoclonal Ig (about 90% containing λ light chains) that forms pathological deposits in critical tissues. Currently there is no explanation for the increased prevalence of t(11;14) in AL. Perhaps there is a novel t(11;14) phenotype that rarely progresses beyond a minimum MGUS tumor mass and usually is not detected by conventional serum electrophoresis, so that it is not detected in the absence of pathological deposits.

MAF TRANSLOCATION GROUP

Tumors with translocations affecting any of the three MAF genes share a very distinctive gene expression profile.³⁵ Many of the genes that are up-regulated in these tumors are thought to be shared targets for all three MAF transcription factors. Notably, these putative targets include *CCND2* and other genes (*ITGB7*, *ARK5*) that appear to affect the phenotype of the tumor cells, and its potential interactions with the bone-marrow microenvironment,^{30,36} although the transcription targets critical for MM pathogenesis have not been determined. The prevalence of c-MAF translocations is lower in MGUS/SMM than in MM.²¹ Although it is assumed that tumors with all three kinds of MAF translocations might have similar phenotypes, including

a very poor prognosis, it is curious that 4/56 (7%) of MGUS/SMM tumors, but only 11/559 (2%) of MM tumors, have MAFB translocations (Kuehl, unpublished).^{14,35}

MMSET/FGFR3 TRANSLOCATION GROUP

The prevalence of this translocation appears to be substantially lower in MGUS/SMM than in MM,^{14,21,32,35,37} although one study reported only a slightly decreased prevalence in MGUS/SMM compared to MM.¹⁸ Although MMSET is dysregulated in all cases, nearly one third of patients with the t(4;14) translocation do not express *FGFR3*. The lack of *FGFR3* expression seems to be related mainly to the loss of der(14), but in some cases der(14) is present and other mechanisms account for the loss of *FGFR3* expression.^{37,38} It is unknown whether the loss of *FGFR3* expression is a primary or a secondary event, or even whether dysregulation of *FGFR3* is critical in pathogenesis. In this regard, it may be significant that the t(4;14) is the only known mechanism that dysregulates MMSET. Although it seems likely that dysregulated *FGFR3* might complement dysregulated MMSET in the early stages of pathogenesis, and even at later stages of pathogenesis in some tumors, there is no clear evidence that unmutated *FGFR3* is important at any stage of pathogenesis. Rare tumors with t(4;14) sometimes can acquire kinase-activating mutations of the dysregulated *FGFR3* during tumor progression, and there is evidence that the survival and proliferation of these tumors is dependent on the mutated *FGFR3*.^{25,29} Other t(4;14) tumors have a K- or N-RAS mutation that seems mutually exclusive of *FGFR3* mutations, which suggests why at least some of these tumors would not require *FGFR3*.²⁹ The apparently invariant dysregulation of MMSET in MM tumors with a t(4;14) suggests a critical role for dysregulated MMSET in the initiation and maintenance of these tumors. In about a third of cases, the t(4;14) translocation results in loss of amino-terminal sequences of MMSET, so that translation must start from an internal methionine.^{24,39} However, there is no convincing experimental data that define the function of MMSET, or indicates how it might contribute to the pathogenesis of these MM tumors.

TWO RECURRENT TRANSLOCATIONS FROM DIFFERENT TRANSLOCATION GROUPS IN THE SAME TUMOR CELL

Rare MGUS or MM tumors, or HMCLs, can have two different recurrent translocations (Kuehl, unpublished).^{6,18} In all of these cases the translocations are from two different translocation groups, with all combinations having been observed. In some of these cases, one of the translocations clearly is secondary, since it is found in only a subset of tumor cells, or is presumptively secondary since it is a complex translocation or insertion. These rare examples suggest that the three different kinds of translocations have the potential to complement one another.

MYC TRANSLOCATIONS: A PARADIGM FOR SECONDARY TRANSLOCATIONS

Translocations that involve a *MYC* gene are rare or absent in MGUS, but occur in 15% of MM tumors, 44% of advanced tumors, and nearly 90% of HMCLs. 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 in only one HMCL. These translocations, often heterogeneous in primary tumors, are usually complex rearrangements or insertions, sometimes involving three different chromosomes.^{6,40-42} An Ig locus is involved in 25%⁴⁰ to 60% (Gabrea, unpublished) of these translocations. The IgH locus is involved somewhat more than the Igλ locus, but the Igk locus is only rarely involved. Thus *MYC* rearrangements are thought to represent a very late 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

interleukin 6 (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 PCL, a surprising result given the high prevalence in advanced primary tumors and HMCLs that are derived from primary and secondary PCL.²⁰ Second, a large study by Avet-Loiseau and colleagues showed no effect of MYC rearrangements on prognosis.⁴³ Unfortunately, they were not able to determine whether MYC/Ig rearrangements affect prognosis. By contrast, in an analysis of 596 patients at Arkansas for which gene expression profiling (GEP) data were 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 (Kuehl, unpublished).

SECONDARY IG TRANSLOCATIONS

Approximately 10-20% of IgH translocations in MGUS and MM do not involve MYC or one of the seven recurrent partners described above, but partner loci have rarely been identified.^{18,19,21,44-46} In contrast to IgH translocations involving recurrent partners, these IgH translocations, as well as most translocations involving IgL loci, share many similarities with MYC translocations: breakpoints not in or near IgH switch or V(D)J regions, unbalanced or complex structures, and occurring with similar frequencies in hyperdiploid (HRD) and non-hyperdiploid (NHRD) tumors (whereas the recurrent or primary translocations occur predominantly in NHRD tumors, see below). Thus, most of them are likely to represent secondary Ig translocations that can occur at any stage of tumorigenesis, including MGUS.^{16,31,44,47}

CHROMOSOME CONTENT SEEMS TO BE ASSOCIATED WITH AT LEAST TWO DIFFERENT PATHOGENIC PATHWAYS

There is a clear consensus that chromosome content reflects at least two pathways of pathogenesis. Approximately half of tumors are HRD (48-75 chromosomes), and typically have multiple trisomies involving chromosomes 3,5, 7, 9, 11, 15, 19, and 21, but only infrequently (<10%) have one of the recurrent IgH translocations. NHRD tumors (<48 and/or >75 chromosomes) usually (~70%) have one of the recurrent IgH translocations.⁴⁷⁻⁵⁰ Tumors that have a t(11;14) translocation may represent a distinct category of NHRD tumors as they are often diploid or pseudodiploid, sometimes with this translocation as the only karyotypic abnormality detected by conventional cytogenetics. In contrast to the selective occurrence of recurrent IgH translocations in NHRD tumors, other genetic events (17p loss or p53 mutations, RAS mutations, secondary Ig translocations, MYC translocations) often occur with a similar prevalence in HRD and NHRD tumors. Extramedullary MM tumors and HMCLs nearly always have an NHRD phenotype, consistent with the hypothesis that HRD tumors are more stromal-cell-dependent than NHRD tumors.^{20,34} We have virtually no information about the timing, mechanism, or molecular consequences of hyperdiploidy. We do not know if the extra chromosomes are accumulated one at a time in sequential steps, or as one catastrophic event. For tumors that are hyperdiploid but have one of the recurrent translocations - most often a t(4;14) - we do not know whether hyperdiploidy occurred before or after the translocation.

LOSS OF CHROMOSOME 13/13Q14 (Δ 13)

About 50% of MM tumors^{19,32,51-53} and 40-50% of MGUS^{18,32,54} tumors have Δ 13 in most tumor cells, suggesting that this is often an early event in pathogenesis. In most cases, Δ 13 represents whole-chromosome monosomy,^{55,56} but in a subset of tumors the common deleted region seems to be located at 13q14,^{46,55-59} although no critical molecular abnormality has been confirmed at this time. The retinoblastoma gene falls within the

minimally deleted region; however, inactivating mutations of the remaining allele are not commonly seen. Haploinsufficiency for RB1 is being investigated as a possible mechanism (Chng, unpublished), and as a target it provides a possible explanation for the observation that $\Delta 13$ is less commonly seen in CCND2-expressing tumors than in CCND1-expressing tumors. ($\Delta 13$ occurs in 80-90% of tumors that have either a t(4;14) or t(14;16) translocation, but in 30-40% of other tumors.)^{21,60} Although CCND1 and CCND2 have largely redundant functional capabilities, CCND1 has been shown to have an additional ability to antagonize RB1, suggesting that CCND2 tumors may be more critically dependent on the level of RB1 protein.⁶¹

GAIN OF CHROMOSOME 1Q21

Using a combination of FISH, array comparative genomic hybridization (aCGH), and GEP, a number of laboratories has determined 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.⁶²⁻⁶⁴ 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.⁶⁵ 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.

ACTIVATING RAS MUTATIONS

The prevalence of activating N- or K-RAS mutations is about 30-40% of newly diagnosed MM tumors, with only a small increase occurring during tumor progression.^{66,67} The prevalence is about 45% in HMCLs.²⁹ Importantly, less than 5% of MGUS tumors have RAS mutations, consistent with the hypothesis that RAS mutations may mark, if not mediate, the MGUS-to-MM transition.^{66,68} Recent studies indicate that the prevalence of RAS mutations is substantially higher in tumors that express Cyclin D1 compared to tumors that express Cyclin D2, with t(4;14) tumors having a particularly low prevalence of RAS mutations.⁶⁸ Although it seems reasonable to speculate that normal FGFR3 minimizes the need for RAS mutations in t(4;14) tumors, there is no solid evidence supporting this hypothesis. Two recent large, unpublished studies differ in that Fonseca et al⁶⁹ find N-RAS and K-RAS mutations, respectively, in 17% and 6% of tumors, whereas M. Kuehl and J. Shaughnessy (unpublished) find N-RAS and K-RAS mutations, respectively, in 14% and 17% of tumors. The latter group also found that the prevalence of N-RAS mutations was substantially higher in tumors that express Cyclin D1, whereas K-RAS mutations occurred with a similar prevalence in tumors that express Cyclin D1 or Cyclin D2. Neither group was able to identify a specific GEP or an effect on prognosis of either N- or K-RAS mutations. We suspect that tumors lacking RAS mutations have alternative oncogenic abnormalities, but we have no experimental evidence for this hypothesis.

ABNORMALITIES OF P53 GENE AND CHROMOSOME 17P LOSS

Mutations of p53 are relatively rare in newly diagnosed MM, occurring in approximately 5% of tumors. However, the frequency of mutations appears to increase with disease stage, and is

about 30% in PCL and 65% in HMCLs.⁷⁰⁻⁷³ Deletion (mainly mono-allelic) of p53, as detected by interphase FISH, occurs in about 10% of MM tumors and approximately 40% of PCL and HMCLs.^{52,74} However, it should be noted that there is no definitive evidence that the critical chromosome 17p loss is *TP53*. Certainly, *TP53* is contained within the minimal deleted region on 17p13 in an analysis of 67 MM patients by aCGH from the Mayo Clinic. Occasionally, these deletions can be quite small, and thus are not always detected by interphase FISH using bacterial artificial chromosome (BAC) probes (Chng et al, unpublished). Although almost all 17p13 deletions detected are mono-allelic, there has been no definitive analysis of *TP53* mutation of the remaining allele in these patients to conclusively implicate *TP53* as the critical gene. In a large study comprising 268 MM patients entered into Eastern Collaborative Oncology Group (ECOG) combination chemotherapy studies only five of 31 (16%) patients with 17p13 deletion have mutation of the remaining *TP53* allele.⁷⁰ However, the use of whole bone-marrow DNA may have resulted in a markedly reduced sensitivity of the study. In a smaller study (24 newly diagnosed MM patients) using purified CD138⁺ plasma cells, no *TP53* mutations were detected, but it is unclear whether these samples also have 17p13 deletion.⁷⁵ Therefore, current evidence does not exclude *TP53* as the critical gene deleted on 17p13. Furthermore, the actual impact of 17p13 mono-allelic deletion on the p53 pathway, and whether other cooperating deregulation - e.g. epigenetic silencing of p53 or increased expression of MDM2 - of various components of the pathway are involved, need to be further clarified.

ACTIVATION OF NFKB PATHWAY

It has been suggested in the past that activation of the NFKB pathway is important in the pathogenesis of MM, but little is known about the prevalence of NFKB activation or mechanisms that cause NFKB activation. Recently a promiscuous array of mutations that result in constitutive activation of the NFKB pathway have been identified in about 20% of patient samples and 20/44 HMCLs. The most common event is inactivating mutation of TRAF3 in 13% of patients. In addition, inactivating mutations of TRAF2, cIAP1/2, and CYLD were identified. Chromosome translocations and amplifications resulting in activation of NFKB-inducing kinase (NIK),⁷⁶ CD40, LTBR, TACI, NFKB1, NFKB2 were also reported.⁷⁷ Although activation of both the canonical and non-canonical pathways is seen, the preponderance of mutations results most directly in increased processing of NFKB2 p100 to p52 (i.e. activation of the non-canonical pathway).

Depletion of NIK with shRNAs directed against NIK results in inhibition of both the classical and alternative NFKB pathways, and also growth inhibition. Half of primary MM tumors have an expression signature of NFKB target genes, with activating mutations identified in less than half of these patients. Presumably either other mutations, or ligand-dependent interactions in the bone-marrow microenvironment, are responsible for the NFKB activation in the remaining patients. Clearly we need to know more about intrinsic and extrinsic mechanisms that activate the NFKB pathway in MM, as this seems to be a potentially important pathway for therapeutic intervention.

DYSREGULATION OF A CYCLIN D GENE: A UNIFYING AND EARLY ONCOGENIC EVENT IN MGUS AND MM

It has been proposed that dysregulation of a CYCLIN D gene provides a unifying, early oncogenic event in MGUS and MM.³⁵ About 25% of MM tumors have an IgH translocation that directly dysregulates a CYCLIN D gene or a MAF gene encoding a transcription factor that markedly up-regulates CYCLIN D2. Although MM tumors with a t(4;14) express moderately high levels of CYCLIN D2, the cause of increased CYCLIN D2 expression remains unknown. Despite the fact that normal BM PCs express little or no detectable CYCLIN D1, nearly 40% of MGUS and MM tumors do not have a t(11;14) but are hyperdiploid, have

multiple trisomies of the eight odd-number chromosomes, and bi-allelically express CYCLIN D1 by a mechanism yet to be determined. Most other tumors express increased levels of CYCLIN D2 compared to normal BM PCs, although the mechanism that causes this is also unknown. Only a small proportion of MM tumors do not express increased levels of a CYCLIN D gene compared to normal PCs, but many of these tumors appear to represent samples that are substantially contaminated by normal cells, and another large fraction of these tumors express little or no RB1, eliminating the necessity for expressing a CYCLIN D gene.

DISRUPTION OF OTHER COMPONENT OF THE RB PATHWAY: P16INK4A, P18INK4B, RB1

Besides CYCLIN D, other components of the RB pathway are also commonly dysregulated in MM. The p16INK4A and p15INK4A genes are methylated in about 20-30% of MGUS and MM tumors, and in most HMCLs.⁴⁴ Two recent studies showed that most MM tumors express little or no p16, regardless of whether or not the gene is methylated.^{78,79} 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,⁸⁰ it remains unclear whether inactivation of p16 is a critical and presumably early event in the pathogenesis of MM.

By contrast, it seems apparent that inactivation of p18INK4C, a critical gene for normal plasma-cell development, is likely to contribute to increased proliferation. There is biallelic deletion of p18 in 30% of HMCLs and nearly 10% of tumors in the highest quintile of proliferation, as determined by an expression-based proliferation index.⁸¹ Forced expression of p18INK4C by retroviral infection of HMCLs that express little or no endogenous p18 substantially inhibits proliferation. Paradoxically, about 60% of HMCLs 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 up-regulated 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 HMCLs, the mechanism (s) by which most HMCLs and proliferative tumors become insensitive to increased p18 levels is not yet understood.

AN UPDATED MODEL FOR THE MOLECULAR PATHOGENESIS OF NON-IgM MGUS AND MM

The current model has been updated from an earlier version⁴⁸ mainly by the inclusion of molecular events that dysregulate the NFκB pathway (Figure 1). As summarized above, there are two pathways of pathogenesis: an NHRD pathway and an HRD pathway. Altogether, there are four early and partially overlapping events for which the precise timing is unknown: IgH translocations mediated mainly by errors in switch recombination or somatic hypermutation in germinal center B cells, hyperdiploidy associated with multiple trisomies, loss of chromosome 13 sequences, and dysregulation of a CYCLIN D gene. Although the increased expression of a CYCLIN D gene may not cause increased proliferation, it may make these cells more susceptible to proliferative stimuli, resulting in selective expansion of cells as a result of interaction with BM stromal cells that express IL-6, IGF-1, or other cytokines. Mutually exclusive mutations of K- or N-RAS (or FGFR3 in tumors with a t(4;14) translocation), secondary MYC translocations, and inactivation of p53 by a variety of mechanisms are progression events. Although the precise timing is unknown, promiscuous mutations that constitutively activate the NFκB pathway occur in approximately half of MM tumors, perhaps facilitating independence from environmental factors that extrinsically activate this pathway at earlier stages of pathogenesis. Secondary chromosomal translocations and other genomic

alterations, epigenetic changes, including methylation of the p16INK4A promoter and other genes, and additional inactivation of the RB pathway (inactivation of p18INK4c or RB1) can occur at all stages of tumorigenesis.

GENETIC CLASSIFICATION SYSTEMS

Recent technological advances have resulted in the ability to assess genomic aberrations at both the DNA and RNA levels in a global fashion. This has resulted in a number of new classifications with biological and clinical relevance.

Translocation and cyclin D (TC) classification³⁵

This classification is based on spiked expression of genes deregulated by primary IgH translocations and the universal over-expression of cyclin D genes by either these translocations or another mechanism. The resultant classification identifies eight groups of tumors: those with primary translocations (designated 4p16, 11q13, 6p21, Maf), those that over-expressed *CCND1* and *CCND2* either alone or in combination (D1, D1&D2, D2), and the rare cases that do not over-express any cyclin D genes ('none'). Most of the patients with HRD MM fall within the D1 and D1&D2 groups.

The advantage of this classification system is that it focuses on different kinds of mechanisms that dysregulate a Cyclin D gene as an early and unifying event in pathogenesis. The underlying cyclin D deregulation potentially has an important therapeutic implication, as differential targeting of cyclin D may be very useful and add specificity to treatment. Indeed, some potential agents targeting cyclin D2 have been identified in a drug library screen (A. K. Stewart, personal communication).

This classification has great potential for translation into the clinic as it involves the measurement of relatively few markers, and most of the translocations can be detected by FISH. In this regard, cut-offs for each gene/probe used for the TC classification has been derived, and a set of criteria for assigning new cases has been formulated (see Appendix).

On the downside, the TC classification does not identify patients with HRD myeloma clearly, with the majority of these patients falling into the D1 and D1&D2 groups. D1&D2 HRD MM appears to have more proliferative disease, but the survival of these patients is not different from those that are D1. In addition, the clinical and biological significance of the D2 group is unclear.

UAMS molecular classification of myeloma

Recently, the group from UAMS (University of Arkansas for Medical Science) derived another MM classification using an unsupervised approach, and identified seven tumor groups characterized by the co-expression of unique gene clusters.⁴² Interestingly, these clusters also identify tumors with t(4;14), maf translocations, t(11;14) and t(6;14), corresponding to the MS, MF and CD-1 and/or CD-2 groups respectively. In this analysis, t(11;14) and t(6;14) can belong to either the CD-1 or CD-2 group, depending on expression of CD20 and other B-cell-related genes. This is consistent with the finding that t(11;14) and t(6;14) have very similar expression profiles, clinical profiles and outcome. In contrast to the TC classification, the UAMS classification identifies HRD MM as a distinct HY group. However, this may be somewhat misleading, since the HY group, which is about 28% of MM tumors (Table 1), includes only about 60% of HRD tumors. The distribution of the remaining HRD tumors among the other six groups has not been clarified, although most are probably in the PR group, defined by increased expression of proliferation-related genes, and the LB group, defined by low bone disease and lower expression of genes associated with bone disease in MM such as FRZB and DKK1.⁸² The PR, MS and MF groups identify patients with poor prognosis. The PR group,

containing patients with t(4;14), t(11;14), and HRD patients, identifies the patients within these categories with more proliferative disease associated with poorer outcome.

The advantage of the UAMS molecular classification is that it is clinically relevant. It identifies the main genetic subtypes and other clinically relevant subtypes such as the high-risk PR subgroup and the CD20-expressing CD-2 group. It is also interesting that an unsupervised analysis of GEP data essentially identifies the main genetic subtypes of MM, suggesting that the predominant transcriptional heterogeneity seen within MM are driven by these pivotal primary genetic events and/or by progression events such as proliferation in the PR group. One of the deficiencies of this classification is that samples with white-cell and normal plasma-cell contamination were excluded from the classification, as the expression signatures from these contaminating cells may affect assignment to the different groups. Furthermore, classification is based on composite expression of large sets of genes. It is therefore uncertain how this can be applied clinically.

Comparison of TC and UAMS molecular classifications

There is significant overlap between the TC classification and the UAMS molecular classification (Table 1). When we compared TC classification of the 414 MM cases assigned a UAMS molecular classification in the original dataset, the MS and MF group correspond to the 4p16 and Maf groups respectively with 100% concordance. The CD-1 and CD-2 groups together correspond to the 11q13 and 6p21 groups (88% concordance). The HY group contains most of the TC D1 and D1&D2 cases (96% concordance). The bulk of the TC D2 cases (67%) fall within the LB group. The PR group contains a mix of patients from the different TC class: 13% 4p16, 4% Maf, 9% 11q13, 21% D1, 19% D1+D2, 28% D2 and 6% None. The PR group is therefore enriched for *CCND2*-expressing tumors, consistent with the hypothesis that these tumors are more aggressive.

In the original paper, this molecular classification was only applied to non-contaminated samples. We applied the same gene classifier for each molecular subgroup, using prediction analysis for microarrays (PAM analysis), to 145 samples with myeloid contamination in the same UAMS dataset (Table 2). The concordance between 4p16 and MS, Maf and MF, D1 or D1&D2 and HY was still excellent. However, in these contaminated samples, the CD-1 and CD-2 groups correspond poorly with 11q13 and 6p21 cases (Table 3). It would appear that the TC classification may be more reliable in contaminated samples, especially in identifying the samples with t(6;14) and t(11;14). It is also clear that the distribution of the molecular subtypes by either classification is skewed, with marked under-representation of the tumors with translocations (MF/Maf, MS/4p16, CD-1+CD-2/6p21+11q13) (Tables 1 and 2). The reason for this is unclear, but it probably explains the significantly better prognosis of patients from whom the contaminated samples are obtained.⁴²

MGUS-like MM

Recently, Zhan and colleagues identified an MGUS signature.¹⁴ When applied to a large dataset of MM patients, about 30% of MM tumors also express this MGUS signature, which they termed MGUS-L MM. They found that MGUS-L-MM has more benign clinical features and, despite lower complete response to treatment, has better survival. Interestingly, in the two datasets examined, MGUS-L MM is enriched for the CD-1 subtypes and was never seen in the PR subtypes. There were, however, some inconsistencies, as there is enrichment for the HY group in one dataset but not the other.¹⁴ It is often difficult to extricate the tumor-cell gene expression signature from contaminating non-malignant cells in the study of minimal plasmacytosis states such as MGUS, even after cell purification. Although the authors try to explain their results in the context of these technical limitations, it is unclear how they define and exclude the effect of contamination. The better survival of patients with MGUS-L MM

adds credence to their biological relevance. However, in their survival analysis, the authors have included contaminated samples, and when we re-analyzed the data with these samples excluded, MGUS-L MM no longer has better survival. In a separate analysis of the dataset, we also found that the MGUS-L MM is enriched for cases over-expressing the gene expression signature of normal plasma cells (e.g. polyclonal immunoglobulin genes). In addition, when we applied the MGUS signature to a cohort of MM patients from the Mayo Clinic with high purity of clonal cells (and no signature of normal plasma cells), none of them express the signature (Chng and Bergsagel, unpublished). Therefore, while compelling, the existence of MGUS-L MM as a biological entity is unclear.

Classification by aCGH

When the abnormalities detected by aCGH were used to cluster MM, two groups of patients corresponding to HRD and NHRD MM could be identified. In addition, HRD MM could be further divided into two groups with significantly different event-free survival but not overall survival (when treated with total therapy II). The main differences between these groups are the enrichment of 1q gain, and chromosome 13 loss in the group with poorer prognosis, and the enrichment of chromosome 11 gain in the group with better prognosis. The NHRD MM can also be split into two groups based on chromosome 1, 8 and 16q abnormalities. However, the subgroups of NHRD MM have similar survival.⁸³

These observations require further validation, as the sample size is small and follow-up relatively short. The importance of chromosome 13 deletion in survival of hyperdiploid patients is inconsistent with the results of two large series.^{43,84} The poor prognostic impact of chromosome 1q gain is well established and is probably mediating most of the difference in survival. The classification of MM by aCGH therefore recapitulates previous FISH findings and does not provide additional prognostic information.

PROGNOSTIC IMPLICATIONS OF GENETICS IN MM

Besides providing insights into the biology of disease pathogenesis and evolution, genetic abnormalities are also powerful prognostic factors in MM.^{44,85} A recent large international study developed a reproducible staging system (ISS staging system) applicable across geographical regions, comprising two routine clinical tests: serum albumin and β_2 -microglobulin.⁸⁶ However, genetic factors were not fully assessed in this study. A large study from the IFM group (Intergroupe Francophone du Myelome), comprising more than 900 patients entered into clinical trials, showed that high-risk genetic abnormalities such as t(4;14) and 17p13 deletion significantly dichotomize survival in each of the ISS stages, showing conclusively that genetic factors are powerful prognostic factors that should be incorporated into routine clinical practice.⁴³

Primary translocations

It is now well established that t(4;14) is associated with adverse prognosis following conventional chemotherapy or high-dose therapy with stem-cell transplant (HDT).^{19,37,43,87-90} The reason for adverse outcome appears to be early relapse, as treatment response is not different from that of other genetic subtypes.⁹¹ An earlier study showed that there is no difference in survival between t(4;14) with or without *FGFR3* expression;³⁷ however, an analysis of a larger dataset treated on total therapy II and III (TTII and TTIII) showed that there is a strong trend towards shorter survival for those who have lost *FGFR3* expression (L. Bergsagel, unpublished). This result needs to be confirmed with longer follow-up. It would fit with the hypothesis that the loss of der(14) is a secondary progression event.

Recently, several studies have shown that treatment with bortezomib overcomes the poor prognosis associated with t(4;14) in both newly diagnosed and relapse patients.^{42,92,93} Furthermore, specific inhibitors of FGFR3, one of the genes deregulated by the translocations, have shown efficacy in vitro and in vivo, and are currently in clinical testing.⁹⁴⁻⁹⁷ For these reasons, t(4;14) should be assessed routinely for all patients, as it provides important prognostic information and offers opportunities to tailor therapy, for example, using a bortezomib-containing regimen rather than high-dose therapy with stem-cell transplant up front.

The prognostic significance of t(14;16) is less well established, as the numbers are often too small for meaningful interpretation. However, existing evidence does suggest that t(14;16), and presumably translocations deregulating other maf genes (*MAFA* and *MAFB*), are associated with adverse prognosis. In a large ECOG study of patients treated with combination chemotherapy, patients with t(14;16) have survival as short as patients with t(4;14).¹⁹ In a GEP study, patients with spiked expression of MAF genes, have significantly shorter survival when treated on both TTII and TTIII.⁴²

Newly diagnosed patients with t(11;14) have better prognosis than patients with the other two aforementioned primary translocations. Early data suggest that this group of patients may particularly benefit from HDT resulting in a significantly better survival compared to all other genetic subtypes.^{33,89} However, recent larger studies have failed to confirm this observation.^{19,43,90,98} In contrast, these patients seem to have inferior survival at relapse. In an analysis of relapse patients entered into the Apex trial, t(11;14) patients have significantly worse prognosis compared to other genetic subtypes, regardless of treatment received (dexamethasone or bortezomib) and despite similar response rate and time from diagnosis to trial entry.⁹⁹ These results suggest that the prognostic impact of the genetic subtypes maybe different at relapse.

Chromosome 1q21 gain

A large study from UAMS establish that 1q21 amplification detected by FISH is a significant and independent poor prognostic factor.⁶⁴ 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.⁶³ 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.⁶³

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.⁶³ 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.

Chromosome 13 deletion ($\Delta 13$)

$\Delta 13$ is one of the first established genetic prognostic factors in myeloma, whether patients are treated with combination chemotherapy or HDT.⁴⁴ One important question thus arising is whether the prognostic importance of $\Delta 13$ is due to its association with poor-risk genetic

subtypes or whether it has intrinsic prognostic properties. Several recent large studies conclusively showed that the former is true. The large IFM study showed that the prognostic value of $\Delta 13$ was entirely dependent on its frequent association with t(4;14) and chromosome 17p13 deletion. In patients lacking these two abnormalities, $\Delta 13$ is not significantly associated with survival.⁴³ Similarly, another study of 260 patients treated with HDT from the Spanish GEM/PATHEMA group also showed that the presence of $\Delta 13$ (using a FISH probe centered on *RB1*) without other FISH-based genetic abnormalities is not associated with adverse prognosis.¹⁰⁰ Furthermore, an analysis of only HRD patients showed that $\Delta 13$ has no prognostic impact in patients of this genetic subtype.⁸⁴ Therefore, there seem to be no role for routine detection of $\Delta 13$ by FISH in clinical practice.

17p13 deletion

The prognostic importance of 17p13 deletion detected by FISH has been demonstrated in several large studies.^{19,43,100,101} The large IFM study confirmed that it is a powerful and independent prognostic factor. In fact 17p13 deletion, together with t(4;14), are the only genetic factors included in their prognostic model.⁴³ A recent analysis from UAMS suggest that the expression of *TP53* is correlated with 17p13 deletion by FISH, and using a cut-off *TP53* expression that identifies 10% of patients with the lowest expression, they found that low *TP53* gene expression is an independent factor associated with poor prognosis.⁷⁵ This supports the possibility that *TP53* is the important gene deleted within 17p13. As mentioned earlier, this issue will need to be resolved in the future. A recent study showed that, despite its rarity, *TP53* mutation is associated with significantly shorter survival. The median survival of patients with *TP53* mutation is only 1.5 years in the ECOG study.⁷⁰

Ploidy

Several studies have consistently shown that HRD MM generally has a better prognosis than NHRD myeloma. Within the latter group, cytogenetically defined hypodiploidy in particular appears to have a very poor prognosis,¹⁰²⁻¹⁰⁴ and is one of the factors used by the Mayo clinic to define high-risk patients.¹⁰⁵

In a recent analysis of GEP data of HRD MM, four reproducible molecular signatures could be identified: one over-expressing cancer testis antigen and proliferation genes; one over-expressing HGF, IL6, SOCS3 and PTP4A3; one over-expressing NFkB genes; while the last signature includes under-expressing genes associated with the first three signatures. Importantly, patients expressing the different signature have different survivals from the group expressing cancer testis antigen, having a median survival of 27 months after diagnosis compared to 'not yet reached' for the group expressing the NFkB signature after a median follow-up of 3 years.¹⁰⁶ A separate study, using aCGH, has identified a group of HRD MM patients with chromosome 1q amplification, 13 deletion and 11 trisomies that have significantly shorter progression-free survival than other HRD MM patients.⁸³ Interestingly, 1q amplification is a common feature of both the high-risk HRD MM groups identified by the GEP and aCGH studies. These studies highlight the presence of molecular and genetic heterogeneity within HRD MM and the importance of defining genetic-subtype-specific prognostic factors. Similar heterogeneity probably exists in other genetic subgroups. For example, in the UAMS classification the t(11;14) can belong to the CD-1, CD-2, or PR groups.

Gene-expression-defined high-risk molecular signature

Recently, a molecular signature that defines high-risk disease was identified using GEP. Using log-rank tests of expression quartiles, 70 genes were linked to early disease-related death. The ratio of mean expression levels of the 51 up-regulated to 19 down-regulated genes defined a high-risk score present in 13% of patients with shorter durations of event-free and overall survival, with a hazard ratio exceeding 4.5. The high-risk score was also an independent

predictor of outcome endpoints in a multivariate analysis that included the International Staging System and high-risk translocations. Interestingly, 30% of these genes map to chromosome 1, with the majority of up-regulated genes mapped to chromosome 1q and down-regulated genes mapped to chromosome 1p. Multivariate discriminant analysis revealed that a 17-gene subset (12 up-regulated) could predict outcome as well as the 70-gene model.¹⁰⁷

We have validated this 17-gene signature in two additional datasets of newly diagnosed (Mayo clinic cohort) and relapse (patients entered into an international phase III bortezomib trial) patients. In both settings it was significantly associated with poorer survival. When analyzing the same dataset from which the high-risk molecular signature was derived, we found that the presence of t(4;14) could further dissect the survival of the high-risk but not the low-risk patients defined by this molecular signature. Combining the use of t(4;14) and the high-risk 17-gene signature, three groups of patients with significantly different survival could be identified, with those having both t(4;14) and high-risk 17-gene score with the shortest survival (Figure 2).

The biological significance of this high-risk signature is not yet fully understood. As mentioned above, it is enriched for chromosome 1 genes. In addition, the up-regulated genes are markedly enriched for proliferation-related genes, and the signature is correlated with that of the proliferation index (r^2 value = 0.63). Yet it is a stronger prognostic factor than both 1q amplification or a gene-expression-based proliferation index that is closely correlated with the slide-based plasma-cell labeling index. Understanding the biological basis of this high-risk signature will aid in the selection of novel therapy for these patients with abysmal survival with currently available therapies.

SUMMARY

The study of genetics has greatly enhanced our understanding of the pathogenesis and underlying biological and clinical heterogeneity of MM. In particular, high-risk genetic subtypes have been identified and shown to have differential benefit for certain therapies. A prime example is that treatment with bortezomib seems to overcome the adverse prognosis following HDT associated with t(4;14). In these patients, one may choose not to administer conventional therapy but to start with novel agents. Therefore, the time has come to incorporate genetic evaluation into everyday clinical practice to guide prognosis and treatment. Indeed, this type of risk-adapted therapy has already been initiated at the Mayo Clinic.¹⁰⁸ At the same time, the advent of global high-resolution and high-throughput genomic technology has allowed greater refinement and understanding of heterogeneity within each genetic subtype of MM. In the future, it would be important to translate this new knowledge into clinically verifiable surrogates such that individual patients can be matched to their specific prognostic category and best treatment option.

Practice points

t(4;14), t(14;16) and 17p13 by FISH should be performed as they identify high-risk patients

conventional karyotyping should also be performed as the test is widely available and the presence of cytogenetic abnormalities detected by this method, especially $\Delta 13$ and hypodiploidy, identifies additional high-risk patients that may not have any of the FISH-defined high-risk genetic abnormalities

Research agenda

establish the timing and sequence of the recurrent genetic abnormalities observed in MM

confirm the clinical significance of the various genetic subtypes in other ethnic groups

study the prognostic impact of genetics in relapse patients and patients treated with novel therapies

incorporate the study of genetic markers into biomarker/pharmacogenomics studies that are linked to clinical trials

identify unique pathways deregulated in high-risk patients in order to identify novel therapeutic targets for these patients

identify the critical genes affected and functional consequences of 1q amplification and 13q and 17p13 deletion so that more specific and effective therapeutic interventions in patients with these common and poor-risk genetic abnormalities can be identified

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APPENDIX

Algorithm for setting-up the TC classification

This algorithm was set up using these two datasets available from GEO: GSE 2688 and GSE 5900. All the MAS 5.0 transformed raw values were downloaded and log-transformed.

Probes used and cut-offs.

Sequence	Gene	Probe ID	Norm cut-off	Control value	Raw cut-off ^a	Assignment
1	FGFR3	204379_s_at	500	22.43	11,215	4p16
2	WHSC1	223472_at	7.0	108.4	759	4p16
3	CCND3	201700_at	7	1380	9660	6p21
4	ITGB7	205718_at	8	2162	17,296	Maf
5	MAF	209348_at	15	375.5	5633	Maf
6	MAFB	218559_s_at	100	112.2	11,220	Maf
7	CX3CR1	205898_at	10	223.1	2231	Maf
8	CCND1	208711_s_at	10	619.1	6191	11q13
9	CCND1	208712_at	0.59 (mean + 2 SD of NPC)	787.4	465	D1 and D1+D2
10	CCND2	200953_s_at	1.95 (mean + 2 SD of NPC)	1640	3198	D2 and D1+D2

^aRaw cut-off = control value for GSM 51111 (median chip value for all samples of 196.9) multiplied by norm cut-off value.

Procedure

1. Rank samples in descending order by normalized expression values of FGFR3 (probe 204379_s_at). All samples with expression above cut-off of 500 are assigned 4p16 (t (4;14)).
2. Rank remaining unassigned samples in descending order by normalized expression values of WHSC1 (probe 223472_at). All samples with expression above cut-off of 7.5 are also assigned 4p16 (these are the samples which have lost the FGFR3 derivative).

3. Rank remaining unassigned samples in descending order by normalized expression values of CCND3 (probe 201700_at). All samples with expression value above cut-off of 7 are assigned 6p21 (t(6;14)).
4. Rank remaining unassigned samples in descending order by normalized expression values of ITGB7 (probe 205718_at). Highlight all samples with expression value above cut-off of 8. Reorder the samples in descending order by normalized expression values of MAF (probe 209348_at). Highlight all samples with expression value above cut-off of 15. Reorder the samples in descending order by normalized expression values of MAFB (probe 218559_s_at). Highlight all samples with expression value above cut-off of 100. Finally reorder the samples in descending order by normalized expression values of CX3CR1 (probe 205898_s_at). Highlight all samples with expression value above cut-off of 10. When samples with expression above cut-offs for these four probes have been highlighted, reorder samples in descending order based on ITGB7 expression. Repeat reordering by MAF, MAFB and CX3CR1 sequentially, each time excluding highlighted samples (above cut-off expression) of the preceding gene. When this is done, look across expression of these four genes. For samples over-expressing (i.e. highlighted) at least a combination of either ITGB7 or CX3CR1 and MAF or MAFB, these are assigned the Maf TC class. For samples over-expressing only one of these four genes, they are also Maf TC class if their macrophage index is less than 6 and CCND2 is also expressed. All Maf samples should have CCND2 expression $g > 6.0$ (raw cut-off 9840).
5. Rank remaining unassigned samples in descending order by normalized expression values of CCND1 (probe 208711_s_at). All samples with expression value above cut-off of 10 are assigned 11q13 (t(11;14)).
6. Rank remaining unassigned samples in descending order by normalized expression values of CCND1 (using the other probe 208712_at, representing the poly-adenylated form). Highlight all samples with expression value above cut-off of 0.59. These samples are considered to express CCND1 compared to normal plasma cells. Reorder these samples expressing low levels of CCND1 by ascending value of CCND2 (200953_s_at). Samples with CCND2 expression above 1.95, are considered to express CCND2 at abnormal levels above that of normal plasma cells. Assign those samples that only over-express CCND1 as D1 TC class whereas those that over-expressed both CCND1 and CCND2 are assigned D1+D2 TC class.
7. Finally, rank the remaining samples in ascending order of CCND2 (probe 200953_s_at) expression. Assign those samples with CCND2 expression above 1.95 D2 TC class, whereas those not over-expressing CCND1 or CCND2 are assigned the 'None' class.

TC class assignment for new case

For each sample, the median value of all probes (SM) is determined from the MAS 5.0 transformed raw values. The normalized raw values ($\text{raw value} \times 196.9/\text{SM}$) of probes listed in the table above are extracted and the assignment algorithm followed to designate the TC class for each new sample:

Sequence	Conditions	Assignment
1	204379_s_at >11,215	4p16
2	204379_s_at ≤11,215 AND 223472_at >759	4p16
3	201700_at >9660	6p21
4	(205718_at >17,296 and/or 205898_at >2231) AND (209348_at >5633 and/or 218559_s_at >11,220)	Maf

Sequence	Conditions	Assignment
5	205718_at >17,296 or 209348_at >5633 or 218559_s_at >11,220 or 205898_at >2231 AND macrophage index < 6 AND 200953_s_at >9840	Maf
6	208711_s_at >6191	11q13
7	208711_s_at ≤6191 AND 208712_at >465 AND 200953_s_at ≤3198	D1
8	208711_s_at ≤6191 AND 208712_at >465 AND 200953_s_at >3198	D1+D2
9	208712_at ≤465 AND 200953_s_at >3198	D2
10	208712_at ≤465 AND 200953_s_at ≤3198	None

Values quoted are normalized raw values. Some samples may fit into two categories.

REFERENCES

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2007;57:43–66. [PubMed: 17237035]
2. Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med* 2006;354:1362–1369. [PubMed: 16571879]
3. Landgren O, Gridley G, Turesson I, et al. Risk of monoclonal gammopathy of undetermined significance (MGUS) and subsequent multiple myeloma among African American and white veterans in the United States. *Blood* 2006;107:904–906. [PubMed: 16210333]
4. Lynch HT, Watson P, Tarantolo S, et al. Phenotypic heterogeneity in multiple myeloma families. *J Clin Oncol* 2005;23:685–693. [PubMed: 15681516]
5. Shapiro-Shelef M, Calame K. Regulation of plasma-cell development. *Nat Rev Immunol* 2005;5:230–242. [PubMed: 15738953]
6. Kuehl WM, Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. *Nat Rev Cancer* 2002;2:175–187. [PubMed: 11990854]
7. Rajkumar SV, Fonseca R, Dewald GW, et al. Cytogenetic abnormalities correlate with the plasma cell labeling index and extent of bone marrow involvement in myeloma. *Cancer Genet Cytogenet* 1999;113:73–77. [PubMed: 10459351]
8. Salmon SE. Immunoglobulin synthesis and tumor kinetics of multiple myeloma. *Semin Hematol* 1973;10:135–144. [PubMed: 4266778]
9. Katzmann JA, Abraham RS, Dispenzieri A, Lust JA, Kyle RA. Diagnostic performance of quantitative kappa and lambda free light chain assays in clinical practice. *Clin Chem* 2005;51:878–881. [PubMed: 15774572]
10. Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC, Drayson MT. Serum test for assessment of patients with Bence Jones myeloma. *Lancet* 2003;361:489–491. [PubMed: 12583950]
11. The International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Haematol* 2003;121:749–757. [PubMed: 12780789]
12. Kyle RA, Therneau TM, Rajkumar SV, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med* 2002;346:564–569. [PubMed: 11856795]
13. Rajkumar SV, Kyle RA, Therneau TM, et al. Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood* 2005;106:812–817. [PubMed: 15855274]
14. Zhan F, Barlogie B, Arzoumanian V, et al. Gene-expression signature of benign monoclonal gammopathy evident in multiple myeloma is linked to good prognosis. *Blood* 2007;109:1692–1700. [PubMed: 17023574]
15. Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. *Nat Rev Cancer* 2005;5:251–262. [PubMed: 15803153]
16. Bergsagel PL, Kuehl WM. Chromosome translocations in multiple myeloma. *Oncogene* 2001;20:5611–5622. [PubMed: 11607813]
17. Pasqualucci L, Neumeister P, Goossens T, et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature* 2001;412:341–346. [PubMed: 11460166]

18. Fonseca R, Bailey RJ, Ahmann GJ, et al. Genomic abnormalities in monoclonal gammopathy of undetermined significance. *Blood* 2002;100:1417–1424. [PubMed: 12149226]
19. Fonseca R, Blood E, Rue M, et al. Clinical and biologic implications of recurrent genomic aberrations in myeloma. *Blood* 2003;101:4569–4575. [PubMed: 12576322]
20. Avet-Loiseau H, Daviet A, Brigaudeau C, et al. Cytogenetic, interphase, and multicolor fluorescence in situ hybridization analyses in primary plasma cell leukemia: a study of 40 patients at diagnosis, on behalf of the Intergroupe Francophone du Myelome and the Groupe Francais de Cytogenetique Hematologique. *Blood* 2001;97:822–825. [PubMed: 11157506]
21. Avet-Loiseau H, Facon T, Grosbois B, et al. Oncogenesis of multiple myeloma: 14q32 and 13q chromosomal abnormalities are not randomly distributed, but correlate with natural history, immunological features, and clinical presentation. *Blood* 2002;99:2185–2191. [PubMed: 11877296]
22. Hanamura I, Iida S, Ueda R, et al. Identification of three novel chromosomal translocation partners involving the immunoglobulin loci in newly diagnosed myeloma and human myeloma cell lines. *Blood* 2005;106Abstract 1552
23. Chesi M, Bergsagel PL, Brents LA, Smith CM, Gerhard DS, Kuehl WM. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two multiple myeloma cell lines. *Blood* 1996;88:674–681. [PubMed: 8695815]
24. Chesi M, Bergsagel PL, Shonukan OO, et al. Frequent dysregulation of the c-maf proto-oncogene at 16q23 by translocation to an Ig locus in multiple myeloma. *Blood* 1998;91:4457–4463. [PubMed: 9616139]
25. Chesi M, Nardini E, Brents LA, et al. Frequent translocation t(4;14)(p16.3;q32.3) in multiple myeloma is associated with increased expression and activating mutations of fibroblast growth factor receptor 3. *Nat Genet* 1997;16:260–264. [PubMed: 9207791]
26. Chesi M, Nardini E, Lim RS, Smith KD, Kuehl WM, Bergsagel PL. The t(4;14) translocation in myeloma dysregulates both FGFR3 and a novel gene, MMSET, resulting in IgH/MMSET hybrid transcripts. *Blood* 1998;92:3025–3034. [PubMed: 9787135]
27. Shaughnessy J Jr, Gabrea A, Qi Y, et al. Cyclin D3 at 6p21 is dysregulated by recurrent chromosomal translocations to immunoglobulin loci in multiple myeloma. *Blood* 2001;98:217–223. [PubMed: 11418483]
28. Hanamura I, Iida S, Akano Y, et al. Ectopic expression of MAFB gene in human myeloma cells carrying (14;20)(q32;q11) chromosomal translocations. *Jpn J Cancer Res* 2001;92:638–644. [PubMed: 11429052]
29. Chesi M, Brents LA, Ely SA, et al. Activated fibroblast growth factor receptor 3 is an oncogene that contributes to tumor progression in multiple myeloma. *Blood* 2001;97:729–736. [PubMed: 11157491]
30. Hurt EM, Wiestner A, Rosenwald A, et al. Overexpression of c-maf is a frequent oncogenic event in multiple myeloma that promotes proliferation and pathological interactions with bone marrow stroma. *Cancer Cell* 2004;5:191–199. [PubMed: 14998494]
31. Gabrea A, Leif Bergsagel P, Michael Kuehl W. Distinguishing primary and secondary translocations in multiple myeloma. *DNA Repair (Amst)* 2006;5:1225–1233. [PubMed: 16829212]
32. Avet-Loiseau H, Facon T, Daviet A, et al. 14q32 translocations and monosomy 13 observed in monoclonal gammopathy of undetermined significance delineate a multistep process for the oncogenesis of multiple myeloma. Intergroupe Francophone du Myelome. *Cancer Res* 1999;59:4546–4550. [PubMed: 10493504]
33. Fonseca R, Blood EA, Oken MM, et al. Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood* 2002;99:3735–3741. [PubMed: 11986230]
34. Hayman SR, Bailey RJ, Jalal SM, et al. Translocations involving the immunoglobulin heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. *Blood* 2001;98:2266–2268. [PubMed: 11568015]
35. Bergsagel PL, Kuehl WM, Zhan F, Sawyer J, Barlogie B, Shaughnessy J Jr. Cyclin D dysregulation: an early and unifying pathogenic event in multiple myeloma. *Blood* 2005;106:296–303. [PubMed: 15755896]

36. Suzuki A, Iida S, Kato-Uranishi M, et al. ARK5 is transcriptionally regulated by the Large-MAF family and mediates IGF-1-induced cell invasion in multiple myeloma: ARK5 as a new molecular determinant of malignant multiple myeloma. *Oncogene* 2005;24:6936–6944. [PubMed: 16044163]
37. Keats JJ, Reiman T, Maxwell CA, et al. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. *Blood* 2003;101:1520–1529. [PubMed: 12393535]
38. Santra M, Zhan F, Tian E, Barlogie B, Shaughnessy J Jr. A subset of multiple myeloma harboring the t(4;14)(p16;q32) translocation lacks FGFR3 expression but maintains an IGH/MMSET fusion transcript. *Blood* 2003;101:2374–2376. [PubMed: 12433679]
39. Keats JJ, Maxwell CA, Taylor BJ, et al. Overexpression of transcripts originating from the MMSET Locus characterizes all t(4;14)(p16;q32) positive multiple myeloma patients. *Blood* 2005;105:4060–4069. [PubMed: 15677557]
40. Avet-Loiseau H, Gerson F, Magrangeas F, Minvielle S, Harousseau JL, Bataille R. Rearrangements of the c-myc oncogene are present in 15% of primary human multiple myeloma tumors. *Blood* 2001;98:3082–3086. [PubMed: 11698294]
41. Shou Y, Martelli ML, Gabrea A, et al. Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. *Proc Natl Acad Sci U S A* 2000;97:228–233. [PubMed: 10618400]
42. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. *Blood* 2006;108:2020–2028. [PubMed: 16728703]
43. Avet-Loiseau H, Attal M, Moreau P, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myelome. *Blood* 2007;109:3489–3495. [PubMed: 17209057]
44. Fonseca R, Barlogie B, Bataille R, et al. Genetics and cytogenetics of multiple myeloma: a workshop report. *Cancer Res* 2004;64:1546–1558. [PubMed: 14989251]
45. Sawyer JR, Lukacs JL, Munshi N, et al. Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. *Blood* 1998;92:4269–4278. [PubMed: 9834233]
46. Sawyer JR, Lukacs JL, Thomas EL, et al. Multicolour spectral karyotyping identifies new translocations and a recurring pathway for chromosome loss in multiple myeloma. *Br J Haematol* 2001;112:167–174. [PubMed: 11167798]
47. Fonseca R, Debes-Marun CS, Picken EB, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. *Blood* 2003;102:2562–2567. [PubMed: 12805059]
48. Bergsagel PL, Kuehl WM. Molecular pathogenesis and a consequent classification of multiple myeloma. *J Clin Oncol* 2005;23:6333–6338. [PubMed: 16155016]
49. Smadja NV, Fruchart C, Isnard F, et al. Chromosomal analysis in multiple myeloma: cytogenetic evidence of two different diseases. *Leukemia* 1998;12:960–969. [PubMed: 9639426]
50. Smadja NV, Leroux D, Soulier J, et al. Further cytogenetic characterization of multiple myeloma confirms that 14q32 translocations are a very rare event in hyperdiploid cases. *Genes Chromosomes Cancer* 2003;38:234–239. [PubMed: 14506697]
51. Facon T, Avet-Loiseau H, Guillemin G, et al. Chromosome 13 abnormalities identified by FISH analysis and serum beta2-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. *Blood* 2001;97:1566–1571. [PubMed: 11238092]
52. Fonseca R, Harrington D, Oken MM, et al. Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (delta13) in multiple myeloma: an eastern cooperative oncology group study. *Cancer Res* 2002;62:715–720. [PubMed: 11830525]
53. Zojer N, Konigsberg R, Ackermann J, et al. Deletion of 13q14 remains an independent adverse prognostic variable in multiple myeloma despite its frequent detection by interphase fluorescence in situ hybridization. *Blood* 2000;95:1925–1930. [PubMed: 10706856]
54. Konigsberg R, Ackermann J, Kaufmann H, et al. Deletions of chromosome 13q in monoclonal gammopathy of undetermined significance. *Leukemia* 2000;14:1975–1979. [PubMed: 11069034]
55. Avet-Louseau H, Daviet A, Sauner S, Bataille R. Chromosome 13 abnormalities in multiple myeloma are mostly monosomy 13. *Br J Haematol* 2000;111:1116–1117. [PubMed: 11227093]

56. Fonseca R, Oken MM, Harrington D, et al. Deletions of chromosome 13 in multiple myeloma identified by interphase FISH usually denote large deletions of the q arm or monosomy. *Leukemia* 2001;15:981–986. [PubMed: 11417487]
57. Dewald GW, Kyle RA, Hicks GA, Greipp PR. The clinical significance of cytogenetic studies in 100 patients with multiple myeloma, plasma cell leukemia, or amyloidosis. *Blood* 1985;66:380–390. [PubMed: 3926026]
58. Sawyer JR, Waldron JA, Jagannath S, Barlogie B. Cytogenetic findings in 200 patients with multiple myeloma. *Cancer Genet Cytogenet* 1995;82:41–49. [PubMed: 7627933]
59. Shaughnessy J, Tian E, Sawyer J, et al. High incidence of chromosome 13 deletion in multiple myeloma detected by multiprobe interphase FISH. *Blood* 2000;96:1505–1511. [PubMed: 10942398]
60. Fonseca R, Oken MM, Greipp PR. The t(4;14)(p16.3;q32) is strongly associated with chromosome 13 abnormalities in both multiple myeloma and monoclonal gammopathy of undetermined significance. *Blood* 2001;98:1271–1272. [PubMed: 11510469]
61. Chang H, Qi X, Trieu Y, et al. Multiple myeloma patients with CKS1B gene amplification have a shorter progression-free survival post-autologous stem cell transplantation. *Br J Haematol* 2006;135:486–491. [PubMed: 16995883]
62. Baker GL, Landis MW, Hinds PW. Multiple functions of D-type cyclins can antagonize pRb-mediated suppression of proliferation. *Cell Cycle* 2005;4(2):330–338. [PubMed: 15684604]
63. Fonseca R, Van Wier SA, Chng WJ, et al. Prognostic value of chromosome 1q21 gain by fluorescent in situ hybridization and increase CKS1B expression in myeloma. *Leukemia* 2006;20:2034–2040. [PubMed: 17024118]
64. Hanamura I, Stewart JP, Huang Y, et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. *Blood* 2006;108:1724–1732. [PubMed: 16705089]
65. Zhan F, Colla S, Wu X, et al. CKS1B, over expressed in aggressive disease, regulates multiple myeloma growth and survival through SKP2- and p27Kip1-dependent and independent mechanisms. *Blood* 2007;109:4995–5001. [PubMed: 17303695]
66. Bezieau S, Devilder MC, Avet-Loiseau H, et al. High incidence of N and K-Ras activating mutations in multiple myeloma and primary plasma cell leukemia at diagnosis. *Hum Mutat* 2001;18:212–224. [PubMed: 11524732]
67. Liu P, Leong T, Quam L, et al. Activating mutations of N- and K-ras in multiple myeloma show different clinical associations: analysis of the Eastern Cooperative Oncology Group Phase III Trial. *Blood* 1996;88:2699–2706. [PubMed: 8839865]
68. Rasmussen T, Kuehl M, Lodahl M, Johnsen HE, Dahl IM. Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition in some plasma cell tumors. *Blood* 2005;105:317–323. [PubMed: 15339850]
69. Fonseca R, Price-Troska T, Blood E, et al. Implications of N-ras and K-ras mutations in clinical outcome and the biology of multiple myeloma. *Blood* 2003;102:113.
70. Chng WJ, Price-Troska T, Gonzalez-Paz N, et al. Clinical significance of TP53 mutation in myeloma. *Leukemia* 2007;21:582–584. [PubMed: 17215851]
71. Corradini P, Inghirami G, Astolfi M, et al. Inactivation of tumor suppressor genes, p53 and Rb1, in plasma cell dyscrasias. *Leukemia* 1994;8:758–767. [PubMed: 8182933]
72. Neri A, Baldini L, Trecca D, Cro L, Polli E, Maiolo AT. p53 gene mutations in multiple myeloma are associated with advanced forms of malignancy. *Blood* 1993;81:128–135. [PubMed: 8417784]
73. Preudhomme C, Facon T, Zandecki M, et al. Rare occurrence of P53 gene mutations in multiple myeloma. *Br J Haematol* 1992;81:440–443. [PubMed: 1390218]
74. Drach J, Ackermann J, Fritz E, et al. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. *Blood* 1998;92:802–809. [PubMed: 9680348]
75. Xiong W, Zhan F, Huang Y, Barlogie B, Shaughnessy JD Jr. TP53 Gene Expression, Correlated with 17p13 Deletion, Is a Significant and Independent Adverse Prognostic Factor in Multiple Myeloma Treated with High-Dose Therapy and Auto-Transplants. *ASH Annual Meeting Abstracts* 2006;108:3394.

76. Annunziata CM, Davis RE, Gabrea A, Lenzi G, Kuehl M, Staudt LM. NF-kappaB-inducing kinase activates NF-KB signaling in multiple myeloma. *Proc Amer Assoc Cancer Res* 2006;47:426.
77. Bergsagel PL, Carpten JD, Chesi M, et al. Promiscuous mutations frequently activate the non-canonical NF-KB pathway in multiple myeloma. *Blood* 2006;108:36a.[Abstract 109]
78. Dib A, Barlogie B, Shaughnessy JD Jr. Kuehl WM. Methylation and expression of the p16INK4A tumor suppressor gene in multiple myeloma. *Blood* 2007;109:1337–1338. [PubMed: 17244692]
79. Gonzalez-Paz N, Chng WJ, McClure RF, et al. Tumor suppressor p16 methylation in multiple myeloma: biological and clinical implications. *Blood* 2007;109:1228–1232. [PubMed: 16840723]
80. Dilworth D, Liu L, Stewart AK, Berenson JR, Lassam N, Hogg D. Germline CDKN2A mutation implicated in predisposition to multiple myeloma. *Blood* 2000;95:1869–1871. [PubMed: 10688850]
81. Dib A, Peterson TR, Raducha-Grace L, et al. Paradoxical expression of INK4c in proliferative multiple myeloma tumors: bi-allelic deletion vs increased expression. *Cell Div* 2006;1:23. [PubMed: 17049078]
82. Tian E, Zhan F, Walker R, et al. The role of the Wnt-signaling antagonist DKK1 in the development of osteolytic lesions in multiple myeloma. *N Engl J Med* 2003;349:2483–2494. [PubMed: 14695408]
83. Carrasco DR, Tonon G, Huang Y, et al. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. *Cancer Cell* 2006;9:313–325. [PubMed: 16616336]
84. Chng WJ, Santana-Davila R, Van Wier SA, et al. Prognostic factors for hyperdiploid-myeloma: effects of chromosome 13 deletions and IgH translocations. *Leukemia* 2006;20:807–813. [PubMed: 16511510]
85. Stewart AK, Fonseca R. Prognostic and therapeutic significance of myeloma genetics and gene expression profiling. *J Clin Oncol* 2005;23:6339–6344. [PubMed: 16155017]
86. Greipp PR, San Miguel J, Durie BG, et al. International staging system for multiple myeloma. *J Clin Oncol* 2005;23:3412–3420. [PubMed: 15809451]
87. Chang H, Sloan S, Li D, et al. The t(4;14) is associated with poor prognosis in myeloma patients undergoing autologous stem cell transplant. *Br J Haematol* 2004;125:64–68. [PubMed: 15015970]
88. Winkler JM, Greipp P, Fonseca R. t(4;14)(p16.3;q32) is strongly associated with a shorter survival in myeloma patients. *Br J Haematol* 2003;120:170–171. [PubMed: 12492597]
89. Moreau P, Facon T, Leleu X, et al. Recurrent 14q32 translocations determine the prognosis of multiple myeloma, especially in patients receiving intensive chemotherapy. *Blood* 2002;100:1579–1583. [PubMed: 12176874]
90. Gertz MA, Lacy MQ, Dispenzieri A, et al. Clinical implications of t(11;14)(q13;q32), t(4;14)(p16.3;q32), and -17p13 in myeloma patients treated with high-dose therapy. *Blood* 2005;106:2837–2840. [PubMed: 15976175]
91. Jaksic W, Trudel S, Chang H, et al. Clinical outcomes in t(4;14) multiple myeloma: a chemotherapy-sensitive disease characterized by rapid relapse and alkylating agent resistance. *J Clin Oncol* 2005;23:7069–7073. [PubMed: 16129840]
92. Chang H, Trieu Y, Qi X, Xu W, Stewart KA, Reece D. Bortezomib therapy response is independent of cytogenetic abnormalities in relapsed/refractory multiple myeloma. *Leuk Res* 2007;31:779–782. [PubMed: 16996589]
93. Mateos MV, Hernandez JM, Hernandez MT, et al. Bortezomib plus melphalan and prednisone in elderly untreated patients with multiple myeloma: results of a multicenter phase 1/2 study. *Blood* 2006;108:2165–2172. [PubMed: 16772605]
94. Paterson JL, Li Z, Wen XY, et al. Preclinical studies of fibroblast growth factor receptor 3 as a therapeutic target in multiple myeloma. *Br J Haematol* 2004;124:595–603. [PubMed: 14871245]
95. Trudel S, Ely S, Farooqi Y, et al. Inhibition of fibroblast growth factor receptor 3 induces differentiation and apoptosis in t(4;14) myeloma. *Blood* 2004;103:3521–3528. [PubMed: 14715624]
96. Trudel S, Li ZH, Wei E, et al. CHIR-258, a novel, multitargeted tyrosine kinase inhibitor for the potential treatment of t(4;14) multiple myeloma. *Blood* 2005;105:2941–2948. [PubMed: 15598814]
97. Trudel S, Stewart AK, Rom E, et al. The inhibitory anti-FGFR3 antibody, PRO-001, is cytotoxic to t(4;14) multiple myeloma cells. *Blood* 2006;107:4039–4046. [PubMed: 16467200]

98. Chang H, Qi XY, Stewart AK. t(11;14) does not predict long-term survival in myeloma. *Leukemia* 2005;19:1078–1079. [PubMed: 15815719]
99. Chng WJ, Mulligan G, Bryant B, Bergsagel L. Survival of genetic subtypes of relapsed myeloma may be modulated by secondary events. *Blood* 2007;109:3610–3611. [PubMed: 17409348]
100. Gutierrez NC, Castellanos MV, Martin ML, et al. Prognostic and biological implications of genetic abnormalities in multiple myeloma undergoing autologous stem cell transplantation: t(4;14) is the most relevant adverse prognostic factor, whereas RB deletion as a unique abnormality is not associated with adverse prognosis. *Leukemia* 2007;21:143–150. [PubMed: 17024116]
101. Chang H, Qi C, Yi QL, Reece D, Stewart AK. p53 gene deletion detected by fluorescence in situ hybridization is an adverse prognostic factor for patients with multiple myeloma following autologous stem cell transplantation. *Blood* 2005;105:358–360. [PubMed: 15339849]
102. Calasanz MJ, Cigudosa JC, Otero MD, et al. Hypodiploidy and 22q11 rearrangements at diagnosis are associated with poor prognosis in patients with multiple myeloma. *Br J Haematol* 1997;98:418–425. [PubMed: 9266942]
103. Fassas AB, Spencer T, Sawyer J, et al. Both hypodiploidy and deletion of chromosome 13 independently confer poor prognosis in multiple myeloma. *Br J Haematol* 2002;118:1041–1047. [PubMed: 12199783]
104. Smadja NV, Bastard C, Brigaudeau C, Leroux D, Fruchart C. Hypodiploidy is a major prognostic factor in multiple myeloma. *Blood* 2001;98:2229–2238. [PubMed: 11568011]
105. Stewart AK, Bergsagel PL, Greipp PR, et al. A practical guide to defining high-risk myeloma for clinical trials, patient counseling and choice of therapy. *Leukemia* 2007;21:529–534. [PubMed: 17230230]
106. Chng WJ, Kumar S, Vanwier S, et al. Molecular dissection of hyperdiploid multiple myeloma by gene expression profiling. *Cancer Res* 2007;67:2982–2989. [PubMed: 17409404]
107. Shaughnessy JD Jr, Zhan F, Burington BE, et al. A validated gene expression model of high-risk multiple myeloma is defined by deregulated expression of genes mapping to chromosome 1. *Blood* 2006;109:2276–2284. [PubMed: 17105813]
108. Dispenzieri A, Rajkumar SV, Gertz MA, et al. Treatment of newly diagnosed multiple myeloma based on mayo stratification of myeloma and risk-adapted therapy (mSMART): consensus statement. *Mayo Clin Proc* 2007;82:323–341. [PubMed: 17352369]

Figure 1.

Disease stages and timing of oncogenic events (see text for further details). The degree of intersection between the overlapping triangles estimates the percentage of each genetic subgroup with coexisting genetic abnormalities. The translocation partners in the IgH translocation (TLC) group are ordered according to increasing frequency of concurrent $\Delta 13$. The different mutations activating the different signaling or cell-cycle regulatory pathways are mutually exclusive, i.e., *RAS* and *FGFR3* mutations always occur in different patients.

*The 8q24 partner referred to here is *MAFA*; *C-MYC* is also located on 8q24, but these are usually secondary IgH TLCs.

Figure 2.

The t(4;14) translocation further dissects the survival of high-risk patients defined by 17-gene high-risk molecular signature. Combination of t(4;14) and high risk defined by the 17-gene model ($\log Q4/Q1 > 0.85$) identifies three groups of patients with significantly different survival (log-rank $P < 0.0001$) in patients entered into total therapy II ($n = 351$): a high-risk group ($n = 16$), defined by presence of $\log Q4/Q1 > 0.85$ and t(4;14), with a median overall survival of 16.7 months; an intermediate-risk group ($n = 45$), defined by the presence of $\log Q4/Q1 > 0.85$ without t(4;14), with a median overall survival of 39.8 months; and a low-risk group ($n = 289$), defined by $\log Q4/Q1 < 0.85$, with a median survival not yet reached. (Gene expression and survival data available from the NCBI Gene Expression Omnibus [GEO] using accession number GSE2658.) For patients with $\log Q4/Q1 < 0.85$, the presence of t(4;14) did not significantly alter survival and is therefore combined for survival analysis.

Concordance between the translocation and cyclin D (TC) classification and the UAMS (University of Arkansas for Medical Science) molecular classification in non-contaminated samples.

Subtypes	4p16	MAF	6p21	11q13	D1	D1+D2	D2	None	All cases
MS	68								68
MF		37							37
CD1			2	22	2		1		27
CD2		1	3	50	4		2		61
HY			1	1	106		2	1	116
LB			1	1	8		39	1	58
PR	6	2	1	4	10		13	3	47
All Cases	74	40	7	78	130	23	57	5	414

Concordance between the translocation and cyclin D (TC) classification and the UAMS (University of Arkansas for Medical Science) molecular classification in contaminated samples.

Subtypes	4p16	MAF	6p21	11q13	D1	D1+D2	D2	None	All cases
MS	6								6
MF		3							3
CD1				4	1	1	2		8
CD2		2	5	1		5	2		15
HY				1	44	7	5	2	59
LB			2	1	4	2	21	3	33
PR		1			6	3	9	2	21
All Cases	6	6	7	7	55	18	39	7	145

Table 3

Comparison of concordance within subgroups between contaminated and non-contaminated samples.

Group comparisons	Concordance Non-contaminated (n = 414)	Contaminated (n = 145)
4p16 v MS	1.00	1.00
MAF v MF	1.00	1.00
D1+D1&D2 v HY	0.97	0.86
6p21+11q13 v CD-1+CD-2	0.89	0.48
D2 v LB	0.69	0.64