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Supplement Article

V. Molecular classification and risk stratification of myeloma

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Introduction

Multiple myeloma (MM) cells are the malignant counterpart of post-germinal centre (GC) long-lived plasma cells (PCs), characterized by strong bone marrow (BM) dependence, somatic hypermutation of immunoglobulin (Ig) genes and isotype class-switch resulting in absence of IgM expression in all but 1% of tumours. However, MM cells differ from healthy PCs because they retain the potential for a low rate of proliferation (1–3% of cycling cells). Virtually every case of MM is preceded by a premalignant PC tumour called monoclonal gammopathy of undetermined significance (MGUS) that, like MM, produces a typical M-spike (almost always non-IgM) by serum protein electrophoresis (SPEP) or free light chain in the urine [1].

Primary IgH translocations are an early oncogenic event shared by MGUS and MM

Translocations involving the immunoglobulin heavy chain (IgH) locus are present in at least half of MM cases and are thought to result from errors during the physiological process of class-switch recombination (CSR) or somatic hypermutation. It is presumed that these translocations represent primary – perhaps initiating – oncogenic events as normal B-cells pass through GCs. These translocations result in dysregulated expression of an oncogene that is juxtaposed to the strong Ig enhancers. The prevalence of IgH translocations increase with disease stage: about 50% in MGUS, 55 to 70% for intramedullary MM, 85% in extramedullary MM and >90% in MM cell lines.

Primary IgH translocations dysregulate three gene groups: CCND, MAF and FGFR3/MMSET

There are three recurrent primary IgH translocation groups in MM: CYCLIN D (11q13-*CYCLIN D1*; 12p13-*CYCLIN D2*; 6p25-*CYCLIN D3*) MAF (16q23-*MAF*; 20q12-*MAFB*; 8q24.3-*MAFA*; MMSET/(FGFR3))-4p16-(*MMSET* in all but also *FGFR3* in 80% of these tumours) (Figure 1). It is thought that CYCLIN D translocations only dysregulate expression of a CYCLIN D gene. By contrast, MAF translocations dysregulate expression of a MAF transcription factor that causes increased expression of many genes, including CYCLIN D2 and adhesion molecules that are thought to enhance the ability of the tumour cell to interact with the BM microenvironment. MMSET is a chromatin-remodelling factor that is overexpressed in all tumours with a t(4;14), whereas about 20% of tumours lack der(14) and FGFR3 expression. It is a histone methyltransferase for H3K36me2, and when overexpressed results in a global increase in H3K36me2 methylation, and a decrease in H3K27me3 methylation, which most likely is the cause of the many changes in gene expression observed in t(4;14) tumours [2,3]. In addition, it recently has been determined that MMSET is important for H4K20 methylation at the sites of double strand DNA breaks [4]. Importantly, loss of MMSET expression alters adhesion, suppresses growth and results in apoptosis of HMCLs, suggesting that it is an attractive therapeutic target [2].

Multiple trisomies are an alternative pathogenetic pathway

There is a consensus that chromosome content reflects at least two pathways of pathogenesis. Nearly half of MGUS and MM tumours are hyperdiploid (HRD), usually with extra copies of three or more specific chromosomes (3, 5, 7, 9, 11, 15, 19 and 21). Strikingly, HRD tumours rarely (~10%) have a primary IgH translocation, whereas nonhyperdiploid (NHRD) tumours usually (~70%) have an IgH translocation. HRD patients seem to have a better prognosis than NHRD patients. Interestingly, in patients with t(4;14) or t(14;16) or t(14;20) or del17p, the presence of one or more trisomies is associated with a substantially better prognosis than absence of trisomies [5].

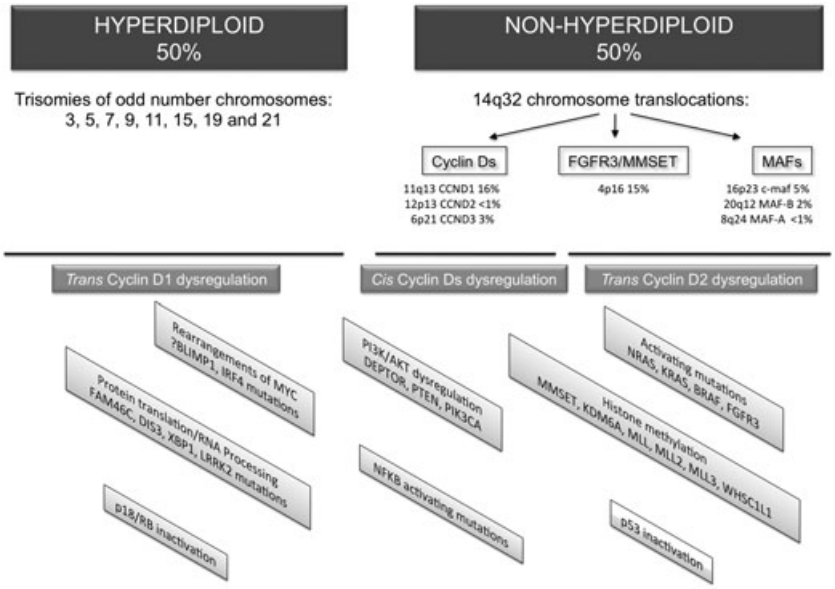

Figure 1. CYCLIN Ds dysregulation in MM

CYCLIN Ds are ectopically expressed throughout MM progression

Almost all cases of plasma cell neoplasm starting from the MGUS stage and independently on the chromosome content aberrantly express one or more of the CYCLIN D genes, and it has been proposed that dysregulation of a CYCLIN D gene provides a unifying, early oncogenic event in MGUS and MM. Although this is not associated with increased proliferation, the expression level of CYCLIN D1, CYCLIN D2 or CYCLIN D3 mRNA in MM and MGUS is distinctly higher than in normal PCs. This results from several mechanisms including a direct *cis*-dysregulation in MM tumours with a CYCLIN D gene translocation or a *trans*-dysregulation in tumours with a translocation of MAF, encoding a transcription factor that directly bind to the CYCLIN D2 promoter. Although MMSET/FGFR3 tumours express moderately high levels of CYCLIN D2, the cause of increased CYCLIN D2 expression remains unknown. The majority of HRD tumours express CYCLIN D1 biallelically, perhaps because they contain a trisomic chromosome 11, whereas most other tumours express increased levels of CYCLIN D2 by unknown mechanism. Only a few percent of MM tumours do not express any CYCLIN D gene, many of which show biallelic deletion of RB1, the cell cycle inhibitor directly targeted by CYCLIN D, therefore bypassing the need for CYCLIN D gene.

Molecular classification of MM

The patterns of spiked expression of genes deregulated by primary IgH translocations and the universal overexpression of CCND genes led to the Translocations and CYCLIN D classification that includes eight groups: (1) those with primary translocations (designated 4p16, 11q13, 6p21, MAF); (2) those that overexpressed CCND1 and CCND2 either alone or in combination (D1, D1&D2, D2); and (3) the rare cases that do not overexpress any CCND genes ('none'). Greater than 95% of tumours in the D1 group are HRD. In addition, most of the patients with HRD MM and trisomy 11 fall within the D1 and D1&D2 groups, whereas those without trisomy 11 fall within the D2 group, although a majority of the D2 group is NHRD. An MM classification is based on an unsupervised analysis of microarray gene expression profiling (GEP) from the UAMS and HOVON-GMMG groups that identified 7 to 10 tumour groups with considerable overlap with each other and with the subgroups of the Translocations and CYCLIN D classification.

Secondary oncogenic events drive MGUS and MM progression

MYC dysregulation. There is increased expression of c-*MYC* in most newly diagnosed MM tumours compared with MGUS tumours [6]. Recently, it was shown that sporadic activation of an *MYC* transgene in GC B-cells in an MGUS prone mouse strain led to the universal development of MM tumours [7,8]. Hence, increased *MYC* expression may be responsible for progression from MGUS to MM. Complex translocations involving *MYC* appear to be secondary progression events that often do not involve Ig loci. They are rare or absent in MGUS, but occur in 15% of newly diagnosed tumours, 50% of advanced tumours and 90% of HMCLs. A recent report

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suggests that a small molecule inhibitor of BRD4 can inhibit *MYC* RNA expression in MM, with therapeutic effect [9].

Chromosome 13 deletion. Chromosome 13 deletion can be an early event in MGUS (e.g. in *MAF*, *MMSET* tumours) or a progression event (e.g. in t(11;14) tumours). A recent genome-wide sequencing study identified mutations of DIS3, a gene of unknown function on 13q, in about 10% of MM.

Activating mutations of *RAS* and *BRAF*. The prevalence of activating *NRAS* or *KRAS* mutations is about 15 to 18% each in newly diagnosed and relapsed MM tumours, but substantially higher in tumours that express *CCND1* compared with tumours that express *CCND2*. For MGUS tumours, the prevalence of *NRAS* mutations is 7%, but *KRAS* mutations have not been described. Recently, *BRAF* mutations were described in 4% of MM tumours [10].

Activating mutations of NF-kappaB pathway. Extrinsic ligands (APRIL and BAFF) produced by BM stromal cells provide critical survival signals to long-lived PCs by stimulating TACI, BCMA and BAFF receptors to activate the NF-kB pathways. Most MGUS and MM tumours highly express NF-kB target genes, suggesting a continued role of extrinsic signalling in PC tumours [11] and at least in part explaining the constant dependency of MM cells on the BM microenvironment. Activating mutations in positive regulators and inactivating mutations in negative regulators of the NF-kB pathway have been identified in at least 20% of untreated MM tumours and ~50% of HMCLs, rendering the cells less dependent on ligand-mediated NF-kB activation and most likely contributing to extramedullary spread of the disease.

Chromosome 17p loss and abnormalities of *TP53*. Deletions that include the *TP53* locus occur in ~10% of untreated MM tumours, and the prevalence increases with disease stage. *TP53* mutations were present in 37% of untreated MM tumours with del17p but not in patients without del17p. Recently, decreased expression of microRNAs miR-199, miR-192 and mir-215 in MM was reported to increase MDM2, an inhibitor of *TP53* [12], contributing to loss of p53 activity.

Gain of chromosome 1q and loss of chromosome 1p. These genomic events frequently occur together in MM, and each of them is associated with a poor prognosis. A relevant gene on 1q is not clear although it may be MCL1, whereas two regions of 1p are associated with a poor prognosis: *CDKN2C* (p18INK4c) at 1p32.3 and *FAM46C* at 1p12.

Other pathogenic events. Secondary Ig translocations can occur at all stages of disease. Mutations in genes regulating RNA metabolism, protein translation and histone-modifying enzymes have also been identified[10].

High-risk MM is associated to intraclonal tumour heterogeneity

Recent evidences suggest that tumour heterogeneity is prevalent in MM, as in many other cancers, and that different subclones are present within the tumour population, characterized by distinct genetic mutations that contributed independently to the tumour progression [13]. The findings suggest a competition between subclones for limited resources and raise the possibility that early, suboptimal treatment may eradicate the 'good' drug-sensitive clone, making room for the 'bad' drug-resistant clone to expand. They support the use of aggressive multidrug combination approaches for high-risk disease with unstable genomes and greater clonal heterogeneity, and sequential one or two drug approaches for low-risk disease with stable genomes and lacking clonal heterogeneity.

Clinical implications of the molecular classification of multiple myeloma

The t(4;14) chromosome translocation is the genetic event in MM with the most important clinical significance. It is a poor prognostic factor for patients treated with alkylating agents, IMiDs and bortezomib. However, there is a survival advantage to the upfront use of bortezomib versus control in these patients, with a suggestion that prolonged use may totally overcome the adverse prognosis [14]. The MAF molecular subgroups, t(14;16) and t(14;20), have each individually been associated with a poor prognosis. In addition, del17p is universally associated with poor prognosis. Finally, patients defined as high-risk by a GEP index of proliferation or other GEP-defined risk scores do poorly. Unlike the t(4;14), for these latter subgroups, neither bortezomib or any other intervention has been shown to offer a survival advantage. On the basis of all of these considerations, the hematologists at the Mayo Clinic have proposed a risk-adapted strategy for the treatment of patients that cannot be enrolled on clinical trials (Figure 2) [15]. The standard risk patients can be treated with lenalidomide and low dose dexamethasone, postponing the toxicity and the inconvenience associated with bortezomib. In contrast, the t(4;14) receive bortezomib as part of induction and maintenance for at least 1 year. Finally, a combination of lenalidomide, bortezomib and dexamethasone with a goal of CR is recommended for the high-risk patients.

Conclusion

Significant progress that has been made helps in understanding the molecular pathogenesis and the biology of MM. Oncogenic pathways can be activated through cell intrinsic or extrinsic mechanisms. Similar to other cancers, MM is characterized by multistage accumulation of genetic abnormalities deregulating different pathways. Much of this knowledge is already being utilized for diagnosis, prognosis and risk stratification of patients. Importantly, from a clinical standpoint, this knowledge has led to the

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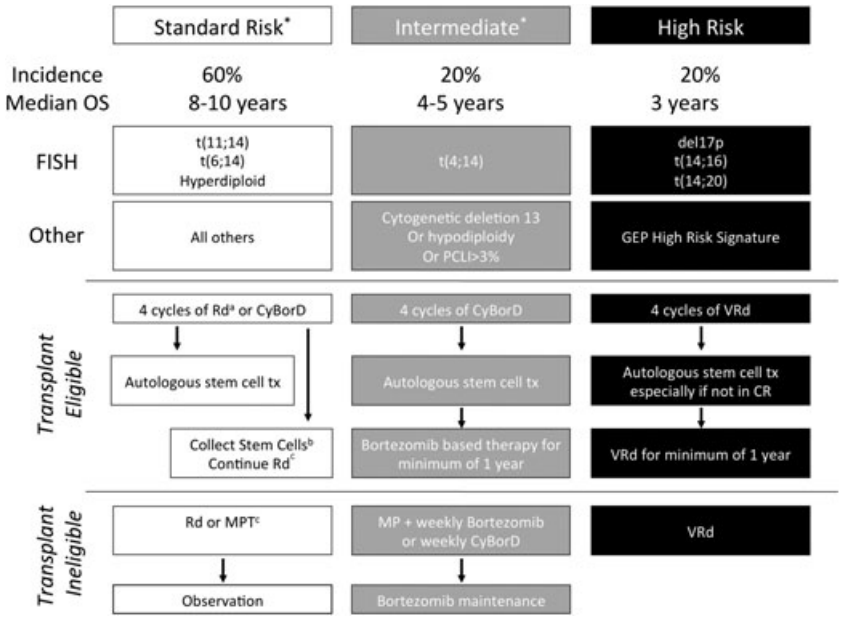

Figure 2. mSMART recommendations for a risk-adapted approach to therapy. * Clinical trials strongly recommended as the first option * Note that a subset of patients with these factors will be classified as high-risk by other factors. a. Bortezomib containing regimens preferred in renal failure or if rapid response needed. b. If age >65 or >4 cycles of Rd, consider G-CSF plus Cytoxan or plerixafor. c. Continuing Rd is optional for patients responding to Rd and with low toxicities; Dex is usually discontinued after first year

development of novel therapeutic strategies, some of which are already in clinical use, and many others showing promise in preclinical and early clinical studies.

Monoclonal gammopathy of undetermined significance and MM karyotypes can be divided into HRD and NHRD on the basis of chromosomal content. Dysregulation of MYC and activating mutations of KRAS are associated with the progression of MGUS to MM. A variety of mutations that affect the pathways shown are associated further disease progression.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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