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The molecular characterization and clinical management of multiple myeloma in the post-genome era

Y Zhou[1,2], **B Barlogie**[1,2], and **JD Shaughnessy Jr**[1,2,3]
[1]Donna D and Donald M Lambert Laboratory for Myeloma Genetics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

[2]Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, Little Rock, AR, USA

[3]Department of Biostatistics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract

Cancer-causing mutations disrupt coordinated, precise programs of gene expression that govern cell growth and differentiation. Microarray-based gene-expression profiling (GEP) is a powerful tool to globally analyze these changes to study cancer biology and clinical behavior. Despite overwhelming genomic chaos in multiple myeloma (MM), expression patterns within tumor samples are remarkably stable and reproducible. Unique expression patterns associated with recurrent chromosomal translocations and ploidy changes defined molecular classes with differing clinical features and outcomes. Combined molecular techniques also dissected two distinct, reproducible forms of hyperdiploid disease and have molecularly defined MM with high risk for poor clinical outcome. GEP is now used to risk-stratify patients with newly diagnosed MM. Groups with high-risk features are evident in all GEP-defined MM classes, and GEP studies of serial samples showed that risk increases over time, with relapsed disease showing dramatic GEP shifts toward a signature of poor outcomes. This suggests a common mechanism of disease evolution and potentially reflects preferential expansion of therapy-resistant cells. Correlating GEP-defined disease class and risk with outcomes of therapeutic regimens reveals class– specific benefits for individual agents, as well as mechanistic insights into drug sensitivity and resistance. Here, we review modern genomics contributions to understanding MM pathogenesis, prognosis, and therapy.

Keywords

gene-expression profiling; array comparative hybridization; multiple myeloma; classification; prognosis

Multiple myeloma: the disease

Multiple myeloma (MM) is a plasma-cell dyscrasia that homes to and expands in the bone marrow, in which it causes a constellation of disease manifestations that includes osteolytic lesions because of osteoblast inactivation and osteoclast activation, anemia, and

Correspondence: Dr JD Shaughnessy Jr, Donna D and Donald M Lambert Laboratory of Myeloma Genetics, Myeloma Institute for Research and Therapy, University of Arkansas for Medical Sciences, 4301 West Markham, Slot 776, Little Rock, AR 72205, USA. shaughnessyjohn@uams.edu.

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immunosuppression because of loss of normal hematopoietic stem cell function and end-organ damage because of excessive monoclonal immunoglobulin secretion;[1] increased bone-marrow angiogenesis is also frequently observed.[2] MM presents with a common histological diagnosis, but it displays enormous genomic complexity as well as marked variations in clinical characteristics and patient survival. To advance treatments, clinical outcome data must be interpreted within the framework of genetic entities, which has proved useful in leukemia and lymphoma,[3–11] and over the past 10 years has contributed to advances in treatment and survival of patients with MM.

For many years, investigations into the molecular lesions driving initiation and progression of MM languished, in part because of the enormously complex karyotypes typically seen in this malignancy. In fact, MM has cytogenetic features more similar to tumors of epithelial origin than to hematological malignancies. Whereas most leukemias and lymphomas present with single chromosomal translocations, karyotypes of myeloma cells from newly diagnosed disease have an average of seven different structural and/or numeric chromosomal abnormalities. This genomic chaos, along with the rarity of the disease, made it difficult to perform the comprehensive correlative studies necessary to identify and better understand the abnormalities involved in initiation and/or progression of the disease and to distinguish nonspecific bystander effects of chromosome instability. Indeed, the first link between a recurrent chromosome abnormality and prognosis was observed only 10 years ago, when deletions of chromosome 13 were associated with aggressive clinical course.[12]

The advent of new technologies, such as interphase fluorescence *in situ* hybridization (FISH), spectral karyotyping, comparative genomic hybridization, single nucleotide polymorphism genotyping, and gene-expression profiling (GEP), has provided the necessary tools to study MM in unprecedented detail. Combining these approaches with maturing technologies, such as high-throughput proteomics, microRNA profiling, and whole-genome sequencing, broadens the spectrum of molecular variables that can be tested, but also poses immense bioinformatics challenges to integrate the massive complexity of these high-dimensional datasets to improve management of MM. This review focuses on the use of GEP of primary disease to classify the disease, define risk, and elucidate underlying mechanisms that are beginning to change clinical decision making and inform drug design.

Studying the complexities of the transcriptome

It is likely that each of the six hallmarks of cancer, outlined in the Hanahan–Weinberg model,[13] ultimately causes or is related to reproducible changes in the expression of subsets of genes within clonal tumor cells and that these patterns are unique and specific to each malignancy. This hypothesis was difficult to test, however, until the completion of the human-genome project[14, 15] and the development of high-throughput tools capable of analyzing the activities of all genes simultaneously.[16] It is now believed that the human genome consists of approximately 25000 mRNA-encoding genes, and this complexity is increased by post-transcriptional modifications, such as alternative splicing.

In the mid-1990s, Brown and coworkers developed a system that used DNA microarrays to monitor the expression levels of thousands of genes in parallel,[16–18] which paved the way for tools that revolutionized molecular biology. The system worked similar to reverse northern blots: cloned DNA fragments immobilized on a solid matrix were used simultaneously to probe mRNA pools from a control source and from the tumor or other tissue of interest, each labeled with a different fluorescent dye (e.g. Cy5 and Cy3). Building on this concept, more advanced high-density oligonucleotide microarrays capable of unprecedented levels of sensitivity and throughput was developed using photolithography and solid-phase chemistry. Now in the industry standard, these whole-genome high-density

oligonucleotide microarrays contain hundreds of thousands of oligonucleotide probes, packed at extremely high densities.[19] The probes are designed to maximize sensitivity, specificity, and reproducibility, which allows consistent discrimination between specific and background signals and between closely related target sequences.[20] Using microarrays for GEP generates large amounts of complex data, demanding equally complex analyses. Indeed, GEP analysis has evolved into a field of its own and in many ways represents a central node in translational research; a comprehensive review of the principles and tools used to analyze microarray data was recently published.[21] Here, we focus on the specific use of microarray profiling in MM, a research that has exploded over the past 10 years.

Microarray technology was first used to study cancer in 1996,[22] and De Vos *et al.*[23] were the first to use GEP to study MM in 2001. In these early experiments, human myeloma cell lines and plasma-cell leukemia samples were analyzed on small-scale, filter-based cDNA arrays to identify genes involved in intercellular signaling. In spite of its small scale, this study revealed that key signaling molecules within the Wnt pathway were altered in MM. Subsequently, Stewart *et al.* used a combination of high-throughput DNA sequencing and microarrays on cells pooled from several cases of plasma-cell leukemia to establish a comprehensive list of genes expressed in MM.[24]

Microarray profiling of MM

On account of the heterogeneous nature of MM growth within the bone marrow, with variable percentages of tumor in a given site as low as 5%, molecular profiling of unfractionated bone-marrow aspirates complicates interpretation of results. To overcome this limitation, researchers have used various means of cell enrichment of plasma cells from bone-marrow aspirates. Plasma cells typically make up <1% of the cells in healthy human bone marrow, so isolation of sufficient numbers of plasma cells from healthy human marrow made large-scale GEP experiments an impractical endeavor for most laboratories. To isolate sufficient numbers of cells for GEP, two different but complementary specialized methodologies were developed. Zhan *et al.*[25] used automated immunomagnetic bead sorting of plasma cells from large-volume bone-marrow aspirates using a monoclonal antibody, BB4, raised against syndecan-1/CD138; this technique routinely has isolated highly homogeneous populations of healthy plasma cells from both bone marrow and tonsil.[26] To create a source of polyclonal plasma cells from healthy donors, Tarte *et al.*[27] developed a method for *in vitro* differentiation of peripheral blood B cells. Global GEP of polyclonal plasma cells and healthy bone-marrow plasma cells derived from immunomagnetic sorting has revealed strong similarities, but also distinct and reproducible differences between the two populations and myeloma cells,[27, 28] suggesting that polyclonal plasma cells may not fully recapitulate the molecular biology of a bone-marrow plasma cell.

Early studies made several contributions to understanding the molecular basis of MM by comparing gene-expression profiles of CD138-enriched plasma cells from the bone marrow of healthy donors and patients with monoclonal gammopathy of unknown significance (MGUS), newly diagnosed MM, and end-stage MM.[25] These studies uncovered potential clues to the molecular pathogenesis of MM—disease-specific changes in gene expression. Myeloma plasma cells can be clearly distinguished from those of healthy donors based on expression of approximately 120 of 6800 genes analyzed. Unsupervised clustering of these early global gene-expression data showed that MM could be divided into four distinct molecular subgroups, MM1–MM4, with MM1 being more similar to MGUS and MM4 being related to myeloma cell lines. The MM4 group also had a higher incidence of cytogenetic abnormalities (CAs) and high serum levels of β-2-microglobulin, clinical features historically linked to poor prognosis. Consistent with these data, genes distinguishing MM4 from the other groups were related to cell proliferation. More advanced

microarray technologies and larger sample sizes have now further divided MM into seven disease classes (discussed below).

These results provided the first evidence that MM is likely numerous molecular entities that presumably use different molecular mechanisms to get to a tumor with a common histology, which has enormous clinical implications. First, the high resolution of molecular classifications allows retrospective evaluation of class-specific efficacy of current therapeutic regimens, which is exceedingly important when designing clinical trials. For example, a new drug might not show a significant effect on a given end point when considering MM as a whole, but the results might be dramatically different if the end point is examined in the context of a particular molecular classification of MM, which might include only 5% of the overall population. Second, identifying the genes whose expression is driving these classes can inform the use of existing agents that might not have been considered and can direct development of new class-specific drugs.

To provide insights into the molecular characterization of plasma-cell dyscrasias and to investigate the contributions of specific genetic lesions to the biological and clinical heterogeneity of MM, Mattioli *et al*. compared the GEP of plasma cells isolated from 7 cases of MGUS, 39 of MM, and 6 of plasma-cell leukemia. MM was heterogeneous at the transcriptional level, whereas MGUS was distinguished from plasma-cell leukemias and the majority of MM cases by differential expression of genes involved in DNA metabolism and proliferation. The clustering of MM cases was mainly driven by the presence of one of five recurrent translocations involving the immunoglobulin heavy-chain (IGH) locus.[29] For example, overexpression of *CCND2* and genes involved in cell-adhesion pathways was observed in cases with t(14;16) and t(14;20), whereas upregulated genes showed apoptosis-related functions in cases with t(4;14). The peculiar finding in cases with t(11;14) was downregulation of the α-subunit of the interleukin-6 receptor (*IL6R*). Finally, cancer-testis antigens were specifically expressed in a subgroup of patients characterized by aggressive clinical evolution of MM.[30]

To further decipher the differences between malignant and normal plasma cells, Jourdan *et al*.[31] recently focused on 58 genes linked with extrinsic and intrinsic apoptotic pathways, caspases, and inhibitor of apoptosis proteins and found B-cell differentiation was associated with change in the expression of pro-apoptotic and anti-apoptotic genes with *TRAIL* being upregulated, whereas *FAS*, *APAF1*, and *BNIP3* were down-regulated in MM cells compared with normal bone-marrow plasma cells.

GEP reveals interactions between MM cells and bone-marrow microenvironment

The bone-marrow microenvironment (BMME) has a critical function in MM cell proliferation, apoptosis, migration, and drug resistance.[32] MM cells in turn disrupt normal bone-marrow homeostasis leading to bone destruction and impaired hemato-poiesis. Clarifying these interactions between the BMME and MM cells is a prerequisite necessary to completely understand MM initiation and progression. GEP has been used to reveal the molecular basis of these interactions.

Corre *et al*.[33] studied bone-marrow mesenchymal stem cells, the only long-lived cells of the BMME, by GEP in patients with MM, patients with MGUS, and healthy subjects. Among the 145 distinct genes differentially expressed in MM and normal bone-marrow mesenchymal stem cells, 46% were classified in the ‘tumor microenvironment’ category according to gene ontology annotations. Genes overexpressed in MM bone-marrow mesenchymal stem cells included known myeloma growth factors such as *IL-6*, amphiregulin, and *IL-1*β, angiogenic factors, and proteins involved in bone disease such as *DKK1*. Pérez-Andrés *et al*.[34] showed different expression profiles of molecules involved in

the interaction with the immunological BMME across MGUS, plasma-cell leukemia, and MM.

MM cells have an affinity for bone, in which they cause osteolytic lesions.[35] Comparing the GEP of MM cells from the MM patients with and without focal bone lesions, Tian *et al.*[36] identified *DKK1*, a Wnt signaling antagonist, as a key gene that was secreted by MM cells and regulates homeostatsis of bone lysis and formation.[37]

Growing evidence indicates a function for increased angiogenesis in MM progression, and markers of angiogenesis have prognostic potential.[2,38,39] Vacca *et al.*[40] used GEP to show an overall induction of *VEGF, FGF-2, HGF-SF, IGF-1*, and IGF-binding protein 3 genes in the endothelial cells derived from MM and MGUS patients compared with human umbilical vein endothelial cell. Hedvat *et al.*[41] compared GEP of MM cells, plasma-cell leukaemia plasma cells, and extramedullary plasmacytoma cells (EPC) and identified several angiogenesis-related genes upregulated in extramedullary plasmacytoma cells, which could confer malignant plasma cells with the ability to grow outside the normal bone-marrow environment. Comparing GEP of genetically identical twin samples, Munshi *et al.*[42] observed increased levels of expression of angiogenesis-related *interleukin-8* (5-fold) and *angiopoietin-1* (5.8-fold) transcripts in MM cells versus healthy twin PCs. Hose *et al.*[43] assessed the expression of 402 angiogenesis-associated genes by GEP in 466 samples, including CD138-purified myeloma cells. They found that although MM cells did not show a significantly higher median number of expressed pro- or anti-angiogenic genes, 97% of MM cell samples aberrantly express at least one of the angiogenic factors: *HGF, IL-15, ANG, APRIL, CTGF*, or *TGFA*.

Heparanase (*HPSE*) degrades *Syndecan-1* and, therefore, enhances tumor cell metastasis,[44] and may have an important function in regulating the growth and progression of MM.[45] Using GEP, Mahtouk *et al.*[46] found that *HPSE* was expressed mainly in the bone-marrow environment from polymorphonuclear cells, T cells, monocytes, and osteoclasts. Bret *et al.*[47] investigated 100 genes involved in synthesis of heparan sulfate (HS) and chondroitin sulfate (CS) chains, which covalently bind to syndecan-1 and are the bioactive components of syndecan-1 in MM cells. Among 100 genes involved in synthesis of HS and CS, 16 were significantly different between normal and malignant plasma cells. Nine of these genes, *EXT2, CHSY3, CSGALNACT1, HS3ST2, HS2ST1, CHST11, CSGALNACT2, HPSE* and *SULF2*, encode proteins involved in glycosaminogly-can-chain synthesis or modifications. GEP data also revealed that syndecan-1 (*SDC1*) is essential for MM cell growth activity of EGF-family ligands in MM.[48]

BAFF and *APRIL* promote survival and growth of MM cells.[49,50] BMME was the main source of *BAFF* and *APRIL* in MM patients.[51] *TACI* is the receptor of *BAFF/APRIL* and its expression level varies in MM patients and is a good indicator of a *BAFF*-binding receptor. Using GEP, Moreaux *et al.*[51] found that MM cells with high *TACI* displayed a mature plasma-cell gene signature, indicating dependence on the bone-marrow environment. In contrast, MM cells with low *TACI* exhibited a gene signature of plasmablasts, suggesting an attenuated dependence on the bone-marrow environment.

To investigate the molecular consequences of myeloma cell interactions with osteoclasts, Ge *et al.*[52] exploited an *ex vivo* culture system and GEP to identify genes whose expression was consistently altered in osteoclasts by coculture with myeloma cells. They identified fibroblast activation protein (*FAP*), a serin protease, as one of 28 genes significantly overexpressed in cocultured osteoclasts and suggested *FAP* as a critical microenvironmental factor and a potential therapeutic target for MM.

GEP reveals universal event in MM is cyclin D dysregulation

Genomic profiling in a large cohort of primary disease revealed that dysregulated expression of cyclin D might be a universal event in myelomagenesis. Relative to plasma cells from the bone marrow of healthy donors, myeloma plasma cells exhibit increased and/or dysregulated expression of either *CCND1*, *CCND2*, or *CCND3*.[53] *IGH*-mediated translocations can directly activate *CCND1* (11q13)[54] or *CCND3* (6p21);[55] *MAF* (16q23)- or *MAFB* (20q11)-activating translocations lead to their transactivation of adhesion molecules and *CCND2*, which is elevated in t(4;14)-positive tumors.[56] Biallelic dysregulation of *CCND1* occurs in nearly 40% of tumors, most of which are hyperdiploid.[53] Elevated levels of *CCND2* and the absence of IGH translocation spikes characterize a novel form of MM discovered through GEP of primary disease (termed 'low bone,' discussed below);[57] interestingly, elevated expression of *CCND2* is not an adverse prognostic factor in this setting.[58]

Validated molecular classification of MM

Using a supervised classification approach that uses prior knowledge of the disease, Bergsagel and Kuehl[29] developed a classification schema based on GEP spikes of the five recurrent translocations, specific trisomies, and expression of cyclin D genes. Reducing the complexity of the microarray from over 50 000 probes to <30 genes, eight translocation/cyclin D (TC) groups were identified. These were termed the 11q13/TC1, 6p21/TC2, 4p16/TC3, maf/TC4, D1/TC5, D1+D2/TC6, D2/TC7, and none/TC8 classes.[53] The authors proposed that these genetic entities are defined by early, perhaps initiating, oncogenic events. The classes exhibited significant, uniform differences in global gene-expression profiles and clinical features, such as prevalence of bone disease, frequency distribution at relapse, and progression to extramedullary tumor growth.[53]

Agnelli *et al*.[59] used this GEP class-prediction model on purified plasma cells from 50 MM cases. The TC1, TC2, TC4, and TC5 groups were characterized by 112 probe sets, but TC3 samples showed heterogeneous phenotypes and no gene biomarkers. The TC2 group, with extra copies of the *CCND1* locus and no IGH translocations or 13q deletion, was characterized by overexpression of genes involved in regulation of protein translation. The failure to validate all TC classes is likely related to the small sample size. Another possibility is that the TC classification is not robust because several of the classes are difficult to classify using this approach; new, different methods of classification are required when dealing with large datasets.

Complementing the supervised approach, unsupervised hierarchical clustering allows samples to self-organize based on underlying correlations in gene-expression patterns (Figure 1). Using a training set of 351 MM cases and a test set of nearly 200 newly diagnosed MM cases, Zhan *et al*.[57] divided MM into seven different reproducible classes (Table 1). These molecular classes, largely consistent with the TC classification, are strongly influenced by distinct gene-expression profiles associated with known genetic lesions, including hyperdiploidy, translocations, cell proliferation, and tumor cell interactions with the BMME.[57]

Four (MF, MS, CD-1, and CD-2) of the seven subclasses are characterized by hyperelevated expression that results from recurrent chromosomal translocations present in approximately 40% of MM, which occur as a result of errors in switch recombination and/or somatic hypermutation.[60] These translocations cause normally silent genes to become juxtaposed with powerful immunoglobulin enhancer elements, resulting in expression 'spikes' readily detectable in microarray studies. **Hy**perdiploid (HY) is characterized by low-level ectopic expression of *CCND1* and overexpression of genes mapping to the odd-numbered chromosomes that typically exhibit trisomy in MM. The LB (low-bone disease) class,

characterized by a low incidence of magnetic resonance imaging (MRI)-defined bone lesions, expresses high levels of *CCND2* and a unique constellation of genes, including *endothelin-1/EDN1*. Unique, reproducible gene-expression programs can define at least six molecular entities; the seventh class of MM, PR (*pr*oliferation), is not related to a primary genetic lesion, but to high expression levels of proliferation-associated genes. This class likely consists of the other classes, but underlying features are masked by expression of proliferation genes. The 700 most differentially expressed genes across seven molecular classes can be found in Supplementary Tables 2 and 3 in Ref.[57]. In the following section, we highlight subsets of these 700 genes thought to be significant in class-specific disease pathogenesis.

MS class

The t(4;14)(p16;q32) translocation characterizes the M**MS**ET (MS) class of MM, which is a high-risk entity that predicts poor prognosis.[61] The t(4;14)(p16;q32) reciprocal translocation results in hyperactivation of both *FGFR3* and *MMSET*/*WHSC1* genes.[62] All t(4;14)-positive disease expresses elevated levels of *MMSET*, but in about 30% of these cases, expression of *FGFR3* is lost.[61,63] As loss of *FGFR3* expression is the only obvious GEP difference between these two types of t(4;14)-positive MM, it seems that *MMSET* has a central function in driving downstream transcriptional events in the MS class. Furthermore, 25% of MM cases in other classes also exhibit upregulation of *MMSET*, supporting its importance in MM pathogenesis.[64] In a comparison of cases with and without t(4;14), GEP studies identified 127 genes as differentially expressed,[64] including *MMSET* and *CCND2*. Notable genes overexpressed in the MS class, relative to other classes, encode *N*-cadherin/CDH2, cadherin-family member desmoglein2/DSG2, Wnt receptors FZ2 and FZD8, and B-cell oncogene PBX1. Underexpressed genes with potential relevance encode adhesion molecules ICAM4, cadherin 7/CDH7, and transcription factor PAX5.

Functional studies suggest that MMSET has a function in cell proliferation by decreasing cell viability and cell-cycle progression, possibly acting through desmoglein 2 (*DSG2*), a cell-surface cadherin molecule involved in cell adhesion and perhaps drug resistance. Thus, desmoglein 2 may be a therapeutic target for MS class MM.[65]

MF class

Accounting for approximately 6% of cases, the ***M***A***F***/***M***A***F***B (MF) class of MM is characterized by the t(14;16)(q32;q23) and t(14;20)(q32;q11) translocations, which result in activation of *c-MAF* and *MAFB* proto-oncogenes, respectively. Cases lacking characteristic *c-MAF* or *MAFB* spikes can be classified as MF, suggesting that other genes of the MAF family may be activated in these cases or that low ectopic expression of either gene is sufficient to drive this classification. Although translocations involving *c-MAF* are seen in <5% of MM cases, *c-MAF* expression is elevated in myeloma cell lines lacking the translocations and in up to 50% of primary samples.[56] These data strongly suggest that *c-MAF* expression may be activated by other mechanisms and attest to the importance of this family of transcription factors in MM pathogenesis. The NF-kB gene-expression signature in the MF class is significantly higher than in the other classes, with the exception of the LB class (see below).[66] It is perhaps noteworthy that TNF-induced *TNFAIP8* is the gene most significantly underexpressed in the MF class, relative to the other disease classes. Clinically, the MF class has relatively low incidence of bone lesions and, consistent with this, has low expression of *DKK1*, a Wnt antagonist produced by myeloma cells and associated with bone disease.[36]

Although mutually exclusive, *MAF*- and *MAFB*-activating translocations induce a common gene-expression signature, suggesting that ectopic expression of the MAF family of

transcription factors results in dysregulation of common downstream targets. In GEP studies aimed at identifying MAFB targets in MM, 284 transcripts were modulated—14 were common to c-MAF and some had functional relationships with MAFB.[67] Additional genes uniquely overexpressed in the MF class that represent known and putative targets of these transcription factors include *NUAK1/ARK5*,[68] *NTRK2*, *ARID5A*, *SMARCA1*, *TLR4*, *SPP1*, and *G6MB6*.

CX3CR1 and *ITGB7* are overexpressed in the MF class,[69] consistent with the report that *CCND2, CX3CR1*, and *ITGB7* are targets of the c-MAF transcription factor.[56] c-MAF–driven expression of *ITGB7* enhanced adhesion of myeloma cells to bone-marrow stroma and increased production of VEGF in these cells, suggesting that MAF can drive myeloma cell adhesion to the extracellular matrix and cellular stroma, resulting in drug resistance and angiogenesis. As expression of *ITGB7* is also elevated in other MM classes, it could be a critical-adhesion molecule in MM that can be activated through multiple mechanisms. Expression of *CCND2* is elevated in other disease classes, but is highest in the MF class. Hurt and coworkers showed that c-MAF transactivated the *CCND2* promoter and enhanced MM proliferation, whereas dominant inhibition of *c-MAF* blocked tumor formation in immunodeficient mice, which highlights the potential significance of this transcription factor in myelomagenesis and the recognized association between MM and activation of one the three *CCND* genes. The *MAF/CCND2* pathway might also interact with therapeutic strategies as it was regulated by *CS1*,[70] a cell-surface glycoprotein to which a therapeutic monoclonal antibody reacts.[71]

CD-1 and CD-2 classes

The t(11;14)(q13;q32) and t(6;14)(p21;q32) translocations, characteristics of the CD-1 and CD-2 classes, directly activate expression of *CCND1* and *CCND3*, respectively. Tumors with *CCND1* and *CCND3* spikes have gene-expression profiles that cluster membership, suggesting that activation of the two cyclin D orthologs results in dysregulation of common downstream transcriptional programs.

Nevertheless, *CCND1* and *CCND3* spikes are associated with two distinct, nonoverlapping gene-expression signatures that were used to distinguish the CD-1 and CD-2 classes. CD-2 is characterized by elevated expression of *CD20/MS4A1, VPREB3*, and *PAX5*—genes expressed in B cells, but normally extinguished in terminally differentiated plasma cells. Of note, CD20 mRNA and protein levels are correlated,[57] but cells expressing elevated *PAX5* mRNA do not express the protein.[72] Unlike CD-2, CD-1 lacks expression of *CD59* (potent inhibitor of complement membrane-attack complex), notch-like protein *NOTCH2NL*, and notch target gene *HES1*. CD-1 is characterized by overexpression of *KLHL4* (a transcription factor), *INHBE*, *FYN* proto-oncogene, *CEBPB/NF-IL6*, and *EVER1* and *EVER2*, two cytoplasmic proteins that colocalize with calnexin, an integral membrane protein of the endoplasmic reticulum (ER).[57]

HY class

Hyperdiploid MM is characterized by trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21. The trisomies are one of two central genetic pathways in development of MM, and this type of disease was earlier shown to have a distinct gene-expression signature.[29] The HY signature is present in nearly 50% of cases and is associated with hyperdiploid karyotypes in >90% of these; however, the signature is also observed in cases that are not identified as hyperdiploid by flow-cytometry analyses. Such cases may arise through a similar initiating genetic mechanism (trisomies of odd chromosomes) with clonal evolution that results in DNA loss from other chromosomes, resulting in a DNA complement that is essentially diploid. Indeed, recent array-based comparative genomic hybridization (aCGH) data

revealed that all molecular classes have at least a partial complement of the common trisomies (our unpublished data).

Genes uniquely overexpressed in the HY class encode *guanine nucleotide-binding protein, gamma 11/GNG11, Trail/ TNFSF10*, Wnt signaling antagonists *FRZB/SFRP3* and *DKK1*, and *MIP1-α chemokine receptor/CCR5*. Overexpression of several interferon-induced genes, including *OAS2, IFI27*, and *IFI35*, is also characteristic of this class. Genes significantly underexpressed in the HY class, relative to the other classes, included *CD52* and genes mapping to chromosome 1q—*TAGLN2*, *CKS1B*, and *OPN3*—whose overexpression has been linked to poor survival.[73] Consistent with these results, integration of aCGH and GEP studies has shown that HY disease rarely exhibits gain of 1q (manuscript submitted). These data are also consistent with aCGH studies that defined disease clusters based on copy-number variation.[74] These studies revealed that the classical hyperdiploid trisomies defined a specific subset of MM that lacks gains of 1q and deletion of chromosome 13.

LB class

A novel class of MM, LB is characterized by low incidence of MRI-defined focal bone lesions and lacks evidence of translocation spikes or HY gene-expression features. Consistent with the absence of MRI-defined focal lesions in the LB class, recent studies integrating positron emission tomography imaging with GEP also revealed that the LB class is uniquely inversely correlated with F18-fluorodeoxyglucose positron emission tomography-defined focal lesion number and intensity.[75]

LB disease is distinguished by overexpression of *endothelin 1/ EDN1*, a soluble factor that is secreted by prostate cancers and that causes osteoblastic metastases of prostate cancer. Interestingly, purified EDN1 induces osteoblast differentiation through suppression of Wnt/β-catenin signaling antagonist *DKK1*,[76, 77] and the LB class is characterized by significantly lower expression of *DKK1*, suggesting that *EDN1* may downregulate *DKK1* in myeloma cells. LB is also associated with high-expression levels of *IL6R*; the MS and MF classes that also have low DKK1 and lower incidence of MRI-defined focal bone lesions, relative to HY, CD-1, and CD-2 classes, share this feature. In contrast to LB, the HY class rarely expresses *IL6R* and expresses high levels of *DKK1* and *EDN1* decoy receptor *EDNRB*. These data suggest a potential connection between *EDN1* signaling and *DKK1* production and bone disease in MM. It is also noteworthy that relative to the other classes, the LB class expresses higher levels of the apoptosis inducing gene *BIK* and the notch target gene and transcriptional repressor *HES5* and lower levels of the chemokine receptor *CCR2, hypoxia-induced Factor 1-a (HIF1A)*, and *SMAD1*, a transcription factor that mediates BMP signaling.

PR class

The PR class is characterized by overexpression of numerous genes related to cell-cycle progression and cell proliferation, including *CCNB2, CCNB1, MCM2, CDCA2, BUB1, CDC2*, and *TYMS*, and also cancer-testis antigen genes, including *MAGEA6, MAGEA3, GAGE1*, and *GAGE4*. Plasma cells from all MM classes have a higher gene-expression–defined proliferation index than plasma cells from healthy donors, but that of the PR class is similar to myeloma cell lines and is significantly higher than non-PR classes. Metaphase CAs, a surrogate for cell proliferation, are present in an extraordinarily high percentage of cases in the PR class. The PR class is associated with poorer survival than other classes, and both hyperdiploid and nonhyperdiploid cases are equally common, with and without concomitant translocation spikes, suggesting that hyperdiploid versus nonhyperdiploid status is likely not sufficient as a sole parameter for risk assessment. Indeed, those cases with both

PR signature and hyperdiploidy are at higher risk than those with the HY signature. Several factors—overexpression of proliferation-associated genes in all non-PR classes, the presence of expression spikes in the PR class, and a shift to the PR class on disease progression—suggest that the PR class is driven by a transformation event secondary to underlying primary genetic lesions.

Classification outliers

In the study by Zhan *et al.*, about 25% of newly diagnosed cases could not be classified because gene-expression signatures of myeloid/lymphoid-lineage cells and/or polyclonal plasma cells predominated and, similar to the PR signature, prevented unsupervised classification. The presence of translocation spikes in these cases supports the idea that this is not a unique class of MM. This contamination signature seems to hold important clinical implications because patients with this signature have lower levels of bone-marrow plasmacytosis, lower incidence of CAs, low β-2-microglobulin and creatinine, and better event-free survival (EFS) and overall survival (OS) than those without the signature.[57]

Using GEP to dissect specific genetic features of MM

GEP and hyperdiploid disease

GEP has been used to further dissect hyperdiploid and nonhyperdiploid disease. A combination of FISH and GEP showed that differential expression of genes involved in protein biosynthesis, transcriptional machinery, and oxidative phosphorylation distinguished the two types of disease.[78] Of 204 genes upregulated in hyperdiploid disease, the majority mapped to the hyperdiploid chromosomes, and 29% of genes upregulated in nonhyperdiploid disease mapped to chromosome 16q;[78] these findings were validated in independent datasets.[78] Consistent with earlier studies,[74] hyperdiploid MM was further divided into two distinct molecular and transcriptional entities, one characterized by trisomy 11 and another lacking this feature, but harboring chromosome 1q gains and chromosome 13 deletes.[78]

Chng *et al.*[79] used GEP to show that hyperdiploid MM is primarily defined by a protein biosynthesis signature driven by a gene-dosage mechanism and to identify four independently validated patient clusters within hyperdiploid MM. One prominent cluster was characterized by cancer-testis antigen, proliferation-associated genes, and higher median plasma-cell labeling index; these patients experienced much shorter survival times than those in the other three clusters. Genes involved in tumor necrosis factor α/TNF-α and *NF-κB* signaling and anti-apoptosis characterized another cluster, and these patients had better responses to bortezomib than those in other clusters. This hyperdiploid disease cluster is probably the same disease entity as the hyperdiploid disease characterized by gain of 1q, lack of trisomy 11, and deletion of chromosome 13, as well as the LB class of MM. Studies integrating aCGH and GEP revealed that the LB class is primarily composed of this novel type of hyperdiploid disease,[80] and the LB class is significantly associated with increased NF-kB activation.[66]

GEP and chromosome 13 deletion

A cohort of MM cases followed for 9 years was used to evaluate the prognostic implications of all individual CAs. Among all CAs and standard prognostic factors examined before therapy, only nonhyperdiploid and deletion of chromosome 13 (del13), alone or in combination, were associated with shortest EFS and OS.[81] A combination of metaphase cytogenetics (to identify CAs), GEP, and interphase FISH (to identify del13) on 146 patient samples showed that overexpression of cell-cycle genes distinguished disease with CA from that without CA; this was especially evident in cases lacking FISH-defined del13.[81]

Interphase FISH evidence of del13 was significantly associated with reduced expression of a subset of genes mapping to chromosome 13, including *RB1*. The authors proposed that haploinsufficiency of genes mapping to chromosome 13, as well as significant upregulation of *IGF-1R* (insulin-like growth factor receptor), may have an amplifying effect on expression of cell-cycle genes, providing a molecular explanation for the dire outcome of patients with del13 compared with those with CA, but lacking del13.

Historically, del13 has been associated with an unfavorable prognosis, but increasing data indicate that its prognostic relevance must be related to the presence of other molecular features. Studies using FISH to detect del13, combined with GEP, on highly purified plasma cells from 80 patients newly diagnosed with MM identified 67 differentially expressed genes, all of which were downregulated in disease with del13. Of these, 44 mapped to chromosome 13, 7 to chromosome 11, and 3 to chromosome 19. del13-positive and -negative cases were differentiated by modulations in global gene expression; in particular, FISH-defined del13 was associated with upregulation of genes mapping to 1q21–1q42 and downregulation of genes mapping to 19p and most of chromosome 11.[82]

GEP and gains of chromosome 1q

Found in up to 45% of patients, abnormalities of chromosome 1 are among the most frequent chromosomal alterations in MM;[83] the short arm is most often associated with deletions and the long arm with amplifications.[74] Gains/amplification of 1q21 increases the risk of MM progression, and incidence of the amplification is higher in relapsed than in newly diagnosed MM.[83,84] GEP studies comparing MM with and without 1q gains[85] identified 61 genes that distinguished the two groups. In cases with 1q gains, 41 of the 43 upregulated genes mapped to 1q12–q44, whereas most of the 18 downregulated genes were localized to chromosomes 13q (7/18) and 11 (6/18). These data suggest that cases with 1q gains typically also harbor del13 and lack trisomy of chromosome 11, features consistent with the LB class. Integrating GEP and DNA copy-number variation data, several independent studies revealed that numerous 1q genes are copy-number sensitive in MM.[74,86]

Gains of 1q are associated with upregulation of genes involved in intracellular protein transport; prominent in the list were *COPA* and *ARF1*, which have functions in vesicle-mediated transport from the ER to the Golgi, and *RABIF* and *RAB3GAP2*, which are related to the Rab GTPases that regulate membrane-vesicle transport. These findings may also partially account for increased expression of genes-encoding proteins involved in energy-production pathways. Genes downregulated in cases with 1q gains include three genes involved in protein translation (*RPLP2, RPL21, and FAU*), which is potentially significant because recent studies suggest that survival of B-cell malignancies (including MM) may be highly dependent on ER– Golgi protein transport, thus targeting this process may be a novel therapeutic strategy.[87]

Cases with 1q gains also showed significantly modulated expression of genes involved in ER stress-induced responses, including upregulation of *CLN3* (chaperone gene), *UBAP2L* and *UBE2Q1* (ubiquitin cycle), *PSMD4* (proteasome degradation), and *CASP4* (initiates apoptosis in response to ER stress).[85] As ER stress-induced apoptosis can have an important function in malignant cells' sensitivity to certain drugs, including bortezomib, these studies suggest that a better understanding of ER stress-induced responses may contribute to important new treatment strategies.[85]

GEP and deletion of 17p13/TP53

A high-risk feature in MM is deletion of 17p13 presumably leads to loss of heterozygosity of *TP53*,[88] a tumor suppressor gene that transcriptionally regulates cell-cycle progression

and apoptosis to modulate cellular responses to DNA damage. Xiong *et al.*[89] found that low expression of *TP53*, seen in approximately 10% of newly diagnosed patients, is highly correlated with FISH-defined *TP53* deletion and inferior clinical outcome and is an independent risk factor. Only a few of the 122 known p53 target genes were highly correlated with *TP53* expression in primary myeloma cells. GEP after ectopic expression of *TP53* in four *TP53*-null-cell lines identified 85 significantly differentially expressed genes—50 upregulated and 35 downregulated. Using these 85 putative target genes, unsupervised hierarchical clustering of myeloma-cell samples from 351 newly diagnosed and 90 relapsed patients revealed two major subgroups that strongly correlated with *TP53* expression and survival. These data suggest that loss of *TP53* expression in MM confers high risk and probably results in deregulation of a novel set of p53 target genes specific to MM and perhaps unique to different cell lineages.[89]

Integrating GEP and high-resolution DNA copy-number analyses

Cigudosa *et al.*,[90] Gutierrez *et al.*,[91] and Avet-Loiseau *et al.*[92] first applied traditional comparative genomic hybridization approaches[93] to expand our knowledge about chromosome instability and copy-number changes in MM. Recently, developed aCGH, similar to GEP, allows simultaneous, high-resolution investigation of copy-number alterations across the entire genome.[94–96] GEP and high-resolution analysis of recurrent copy-number alterations defined 87 discrete minimal common regions within recurrent, highly focal copy-number alterations;[74] unsupervised classification using nonnegative matrix factorization uncovered four subtypes of disease and two subtypes of hyperdiploid disease. One hyperdiploid subtype had classic features—trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19, and 21—whereas the other, associated with inferior survival,[74] lacked trisomies of chromosomes 7 and 11 and also harbored deletion of chromosome 13 and gains of 1q. Another subtype was characterized by amplification of 1q21 and deletion of 1p, suggesting a relationship between this group and GEP-defined high risk. Indeed, a recent analysis of 92 additional cases revealed that copy-number alterations in chromosome 1q and 1p are highly correlated with gene-expression changes that are strongly correlated with risk of death from disease progression, gene-expression–based proliferation index, and 70/17-gene model of high risk (discussed below, *GEP and risk stratification*).[80]

High-density single nucleotide polymorphism microarrays of genomic DNA in the KMS-26 myeloma cell line showed that an amplicon within an unstable chromosomal region (17p11.2-p12) was characterized by a large number of low-copy repeats that mediate deletion and duplication in several genomic disorders and amplifications in solid tumors. Combining this data with GEP and FISH mapping narrowed the region of interest to the *TNFRSF13B/TACI* gene, an important receptor in B-cell development.[97]

In a FISH study of over 800MM cases, deletion of 16q was identified in 19.5% of cases, was associated with poor outcome, was an independent prognostic marker, and conferred additional adverse survival in cases with t(4;14) and/or del(17p).[98] GEP and single nucleotide polymorphism-mapping arrays revealed loss of heterozygosity at 16q12, mapping near *CYLD*, and at 16q23, near *WWOX*. Cases with low expression of *CYLD*, a negative regulator of the NF-kB pathway, defined a ‘low-*CYLD* signature.’ Cases with 16q loss of heterozygosity or t(14;16) had significantly reduced expression of *WWOX*, a tumor suppressor gene involved in apoptosis that lies at the t(14;16) break point.[98]

GEP and risk stratification

Although most cases of MM initially respond to treatment, a subset exhibits resistance to therapy from the outset, and most will develop resistance over time. Therefore, long-term survival in patients with MM can vary considerably, and it is difficult to predict outcome

based on current laboratory tests. High-risk MM is routinely defined by laboratory parameters alone or in combinations as in the Durie–Salmon staging system[99] and International Staging System (ISS).[100] A staging system based on cell morphology, the Bartl grade, has also been developed,[101] and the presence of abnormal metaphase or interphase genetics,[102] high plasma-cell labeling index,[103] and a recently defined flow-cytometry–based test on minimal residual disease are also used.[104] Importantly, the molecular mechanisms by which cells develop resistance are not yet known, but understanding the mechanisms underlying the disease escape from initial drug responsiveness will contribute to more robust prognostic strategies.

To determine whether GEP might provide a better measure of risk stratification, microarray data was correlated with outcome in two independent cohorts, permitting identification and validation of a high-risk gene-expression signature present in approximately 15% of newly diagnosed disease[73] (Figure 2). The high-risk signature is evident in a subset of all molecular classes and negatively influences regardless of class—for example low-risk MS disease fares much better than high-risk MS disease. The '70/17-gene model' of high risk is based on expression patterns of 70 genes, reducible to 17 genes—predominately increased expression of genes from the q arm and reduced expression of genes from the p arm of chromosome 1[73]—which was confirmed by whole-genome microarrays and high-resolution comparative genomic hybridization (our unpublished data).

When subjected to multivariate analysis including the ISS and a gene-expression–based proliferation index, the 70/17-gene model remained a significant predictor of outcome. Improving on risk stratification provided by the ISS, Mulligan *et al.*[105] used U133A microarray data to develop response and survival classifiers for relapsed disease treated with single-agent bortezomib or high-dose dexamethasone that were significantly associated with outcome; a modified version of the 70/17-gene model also predicted poor outcome in relapsed disease.[106] U133A data from newly diagnosed disease validated the 70/17-gene model, but also showed that the t(4;14) translocation remained a significant variable for poor outcome.[107] In addition to its ability to predict the outcome of newly diagnosed MM patients, a recent study showed that the 70-gene model was also an independent and the most significant prognostic factor in an analysis of post-relapse survival in relapsing MM.[108]

Decaux *et al.*[109] recently used a custom cDNA microarray to define a 15-gene model of high risk related to cell proliferation, with a hyperdiploid signature being related to a better survival. Multivariate analysis comparing the 70/17-gene model with the 15-gene model revealed that the 70/17-gene model was significant in all datasets tested, but the 15-gene model was significant in bortezomib trials only. These data, together with unpublished studies, suggest that the 70/17-gene model captures more outcome variability than models or indexes of cell proliferation, considerably improving standard measures; however, it must be noted that the R^2 is only ~30%.

Conversion of GEP low to high risk at relapse

GEP on 71 paired diagnostic and relapse samples indicated increased 70/17-gene model scores in 80% of cases, and this conversion from low to high risk severely impacted post-relapse survival in 14 of 24 cases (58%) (Figure 3). This quantifiable increase in the high-risk score over time, combined with the report that percentages of cells with gains of chromosome 1q increased over time,[73] suggests expansion of a dominant clone with survival and/or proliferation advantages. The almost universal increase in this risk score during disease evolution suggests that evaluating minimal residual disease may benefit from monitoring this traceable molecular signature, in combination with flow-cytometry–based

surrogate. An urgent task is to determine whether a specific baseline GEP signature can prospectively identify which low-risk cases will convert to high risk at relapse.

GEP and the centrosome index

The mechanisms underlying aneuploidy in MM are unclear, but centrosome amplification has been implicated as the cause of chromosomal instability in a variety of tumors and may be involved in MM. Immunofluorescence staining detected centrosome amplification in 67% of monoclonal gammopathies.[110] A GEP-based centrosome index (CI) was created from gene expression of centrosome proteins, which correlated well with immunofluorescence-detected centrosome amplification. High CI (>4) was associated with poor prognostic genetic features and was an independent prognostic factor in a small cohort of heterogeneously treated cases of MM.[110] Prognostic significance of the CI was subsequently validated in two large cohorts of patients entered into clinical trials, showing that a high CI is a powerful independent prognostic factor in both newly diagnosed and relapsed patients, whether treated by intensive therapy or novel agents.[111] Human myeloma cell lines with higher CIs are more responsive to treatment with a novel aurora kinase inhibitor, suggesting the potential for aurora kinases as novel therapeutic targets for patients with poor prognoses.[111]

GEP and CD200

Elevated expression of CD200 is an additional prognostic marker that emerged from GEP studies as a high-risk feature in MM.[112] CD200 is a membrane glycoprotein that imparts an immunoregulatory signal through CD200R, leading to suppression of T-cell–mediated immune responses.[113] CD200 expression was predictive for EFS independent of ISS stage or β-2-microglobin serum levels,[112] but it has not yet been validated in independent datasets or evaluated in the context of OS and all molecular subtypes and models. As failure of immune surveillance may account for MM progression, CD200 modulation might be an important adjunct to cellular immunotherapy. As a cell-surface protein, CD200 is a potential target for monoclonal antibody therapy, but such strategies will have to consider off-target effects and the critical function of this molecule in immune regulation.

Using GEP to understand signaling in disease and its response to therapy

GEP and cancer-testis antigen expression in MM

Cancer-testis antigens are expressed in testis and malignant tumors, but rarely in nongametogenic tissues, which makes them attractive targets for cancer vaccination approaches. GEP studies determined that patients newly diagnosed with MM expressed variable numbers of cancer-testis genes (98% expressed at least one, 86% at least two, and 70% at least three) and that expression of six or more cancer-testis genes was associated with shorter EFS.[114] Ten cancer-testis genes are desirable for circumventing tumor escape mechanisms, and GEP could be useful in identifying which antigens should be used to vaccinate a given patient.[114] Additional independent studies confirmed the association of cancer-testis antigen expression and poor outcome in patients with MM.[115,116] Global GEP studies showed that cancer-testis antigen NY-ESO-1 could be an ideal tumor target antigen for immunotherapy of patients with MM. NY-ESO-1 expression was higher in tumor cells from patients with CAs than in those with no CAs,[117] and NY-ESO-1 expression was significantly higher in cases of relapsing MM, especially in patients with CA.

GEP and IGF signaling

IGF-1R have been implicated in cancer pathophysiology, and *IGF-1R* is universally expressed in various cells of hematologic malignancies (i.e. MM, lymphoma, leukemia) and solid tumors. Specific *in vitro* inhibition of IGF-1R with neutralizing antibody, antagonistic

peptide, or selective kinase inhibitor (NVP-ADW742) has activity against diverse tumor-cell types (particularly MM), even those resistant to conventional therapies. Global transcriptional profiles also delineated pleiotropic antiproliferative/pro-apoptotic molecular sequelae— specifically, modulated intracellular concentrations of key components of these pathways, including Akt, Raf, and IKK. Therapies with NVP-ADW742 or GSK1904529A, alone or in combination with cytotoxic chemotherapy, had significant antitumor activity in an orthotopic xenograft MM model, providing *in vivo* proof of principle for therapeutic use of selective IGF-1R inhibitors.[118,119] Sprynski *et al.*[120] showed that an IGF-1 autocrine loop promoted survival in CD45-negative MM cell lines, whereas CD45-positive cells required addition of either IL-6 or IGF-1. GEP analysis in primary disease revealed that elevated expression of IGF-1R and IL6R conferred an adverse prognosis. High expression of both IGF-1R and IL6R is seen in the MS class, but elevated IGF-1R expression is also seen in non-MS classes and is associated with a poor prognosis. Combining IGF-1–targeted therapy with anti-IL-6 therapy could be promising in the subset of patients with myeloma cells that express IGF-1R.

Pharmacogenomics of short-term in vivo exposure can reveal drug efficacies and mechanisms of action

Mechanisms of cancer cell resistance to chemotherapy are poorly understood, and efficacy measures typically rely on clinical outcome data; however, GEP studies potentially can delineate mechanisms and identify novel strategies for avoiding or overcoming drug resistance, which is a major hurdle for MM therapy and cure. Marton *et al.*[121] and Gray *et al.*[122] were the first to use microarrays to discover targets and effects of therapeutic agents in yeast, and Cheok *et al.*[123] first revealed gene-expression patterns in drug responses of human cancer.

GEP has been used to study drug responses of MM cells *in vitro*. It was first used to identify genes regulated by two FDA-approved drugs, dexamethasone and bortezomib, and genes that may confer drug resistance in MM cells.[124–126] Numerous other studies have since been reported. The Dana–Farber group used GEP to identify the transcriptional signatures of a bunch of distinct drugs in MM celles, such as 2-methoxyestradiol (2ME2), an estrogen derivative,[127] two histone deacetylase inhibitors, SAHA and VPA,[128, 129] IGF-1R) inhibitors,[118] a IL-6 receptor inhibitor, Sant7,[130] an inhibitor of Wnt signaling pathway, PKF115–584,[131] ribonucleotide reductase inhibitor, Didox,[132] and Atiprimod, a oral agent with anti-inflammatory properties.[133] Using GEP, the transcriptional signatures of cellular responses to 5-aza-2′-deoxycytidine and trichostatin,[134] a HSP90 inhibitor, 17-AAG,[135] and a marine-derived compound, Zalypsis,[136] have recently been described.

Recently, GEP has been successfully used to study drug response of MM patients *in vivo*. By comparing GEP of myeloma cells before and 48 h after single-agent therapy with dexamethasone, thalidomide, or lenalidomide, Burington *et al.* found that genes differentially expressed after therapy were prognostic for EFS and OS and were enriched for genes involved in oxidative stress reactions and actin cytoskeleton rearrangements.[137] Remarkably, gene expression altered by thalidomide in newly diagnosed disease and associated with subsequent survival was also altered by lenalidomide, a thalidomide analogue, and the changes were associated with EFS in a salvage trial of patients with relapsed disease.[137] This finding strongly suggests that these genes are powerful biomarkers, and the similar acute gene-expression responses to two related chemotherapeutic agents may provide important insights into the drugs' potential mechanism(s) of action. These results also highlight the similar acute molecular responses to chemotherapies in both primary and refractory diseases.

GEP studies after therapy with proteasome inhibitor bortezomib in 142 newly diagnosed symptomatic MM cases identified 113 genes with significantly altered expression—predominately downregulated proteasome genes—seen in tumor cells from 76% of patients.[138] The post-bortezomib gene-expression signature was associated with a 3-year survival estimate of >80%, which dramatically contrasts a median survival of <24 months in those with activated proteasome genes.[138] Multivariate analysis showed that the post-bortezomib score was an independent predictor of outcome that alone accounted for >50% of outcome variability.[138] These data implied that the activation status of proteasome genes in tumor cells after short-term proteasome inhibition is associated with significant outcome differences in patients with MM receiving polychemotherapy that includes bortezomib.[138]

Conclusions

Using high-throughput genomic analyses and data-mining techniques, a complete landscape of MM molecular pathogenesis is emerging, and powerful validated prognostic models have been developed. Unsupervised clustering of GEP data of large patient cohorts also have revealed that MM heterogeneity can be accurately cataloged and disease classes defined. Importantly, the improved survival observed in specific classes through the use of new treatments, such as thalidomide and bortezomib, buttress the concept of personalized treatment approaches.

Large-scale gene-expression data and large cohorts of uniformly treated patients with long follow-up times have provided more precise and independent prognostic models for stratifying patients with MM. Investigating GEP changes between baseline and relapse has shed light on the mechanisms underlying MM progression and the nearly universal development of multidrug-resistant MM. Pharmacogenomics studies comparing gene-expression profiles at diagnosis and after short-term single-agent therapy have identified genes associated with drug responses, contributing to mechanistic understanding. This is critical for improving existing therapies with personalized treatments and for informing research and discovery of new therapeutics.

It is well known that MM growth and survival are highly dependent on interactions with the BMME, and GEP has uncovered many molecular details of these interactions, which might prove to be the Achilles heels of MM. A prominent example was the use of GEP and MRI imaging of bone to learn that myeloma cells aberrantly synthesize DKK1, a potent inhibitor of Wnt/β-catenin signaling, which is required for osteoblast differentiation and function. These data provided a potential underlying mechanism for the unique MM osteolytic bone disease characterized by complete loss of osteoblast function. An inhibitor of DKK1 is in early Phase I/II clinical trials, attesting to the translational potential of GEP studies. GEP of whole-bone biopsies that contain tumor cells and all the accessory cells are also beginning to reveal details of MM pathogenesis and potential therapeutic targets.

After 10 years of applying GEP to thousands of patient samples in numerous institutions, GEP is emerging from the research laboratory as a clinical tool with the potential for transforming routine management of MM. Although the majority of MM patients can anticipate long-term disease control through a variety of treatment approaches, patients with molecularly defined high-risk disease do not benefit from current approaches. To address this, clinical trials designed to reduce toxicities in low-risk disease and to test new treatment strategies in high-risk disease are underway. When routinely available, molecular-based classification and risk stratification will meet their potential to shift strategies for MM treatment and cure.

We have made every effort to reference as much of the literature on the molecular characterization of myeloma as possible. However, because of size limitations and the rapidly growing body of work, it is likely that we have failed to include some of the works of our coworkers. We apologize for this oversight.

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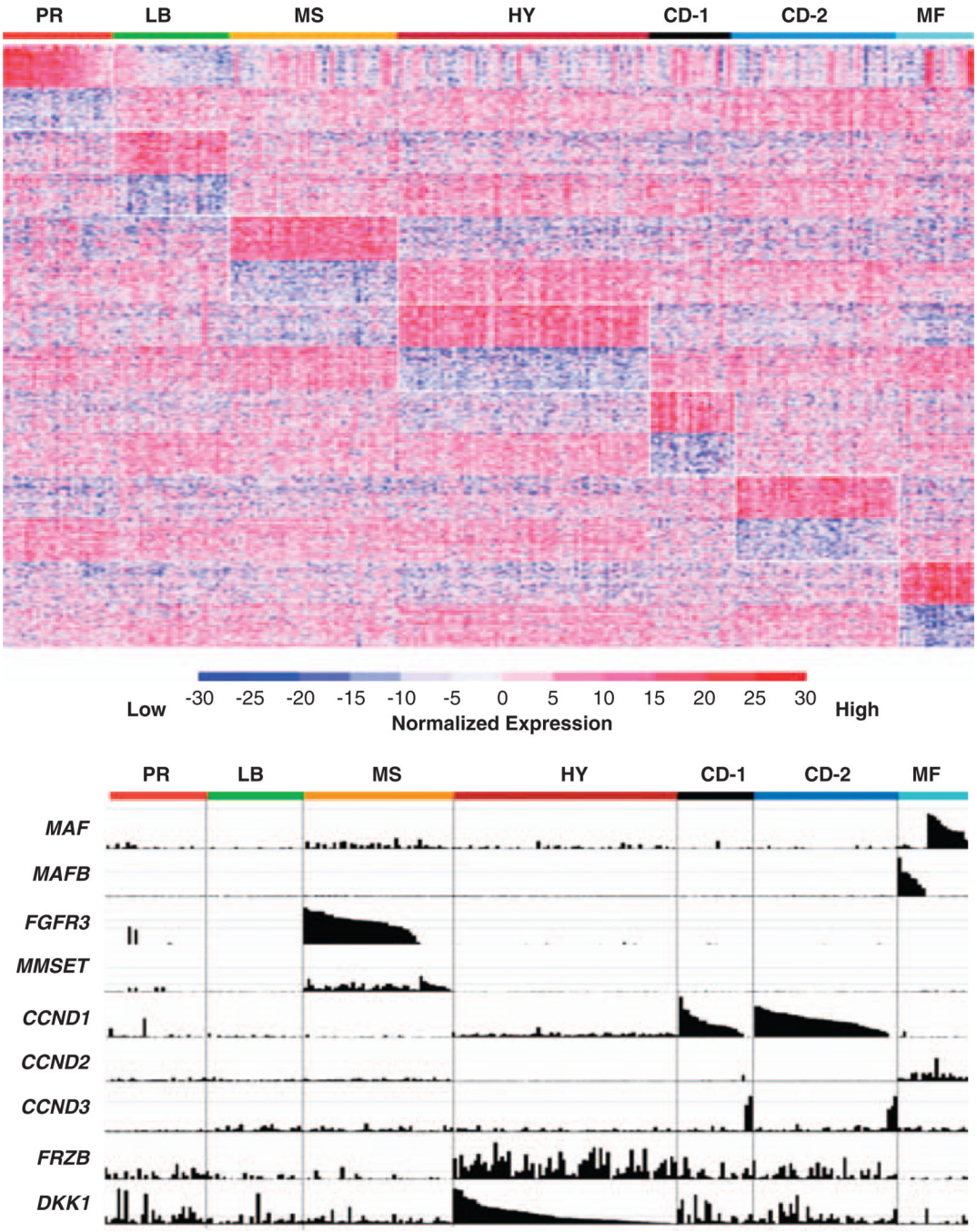

Figure 1.

Classes are characterized by unique GEP patterns. (upper panel) A supervised clustergram of the expression of 700 genes (50 SAM-defined overexpressed and 50 underexpressed genes from each of the 7 classes) across 256 newly diagnosed cases. Genes are indicated along the vertical axis and samples on the horizontal axis. The normalized expression value for each gene is indicated by a color, with red representing high expression and blue representing low expression. (lower panel) The Affymetrix gene-expression signal (expression level: vertical axis) for the mRNA of *MAF MAFB, FGFR3, MMSET, CCND1, CCND2, CCND3, FRZB*, and *DKK1*, within classes presented in the upper panel, are indicated. The normalized expression level for each gene across the samples is given by the

height of each bar. Note that spiked expression of *CCND1, MAF* and *MAFB*, and *FGFR3* and *MMSET* is strongly correlated with specific subgroup designations. Also note that cases retaining the *MMSET* spike, but lacking *FGFR3* spikes maintain similar cluster designation, and *MAF* and *MAFB* spikes cluster in the same subgroups. Several *MMSET* and *CCND1* spikes cases are evident in the PR class. *CCND3* expression is mutually exclusive of *CCND1* expression. Although overexpressed in the HY subgroup, *FRZB* and *DKK1* are significantly underexpressed in LB and MF. Figures reproduced with permission from *Blood*.

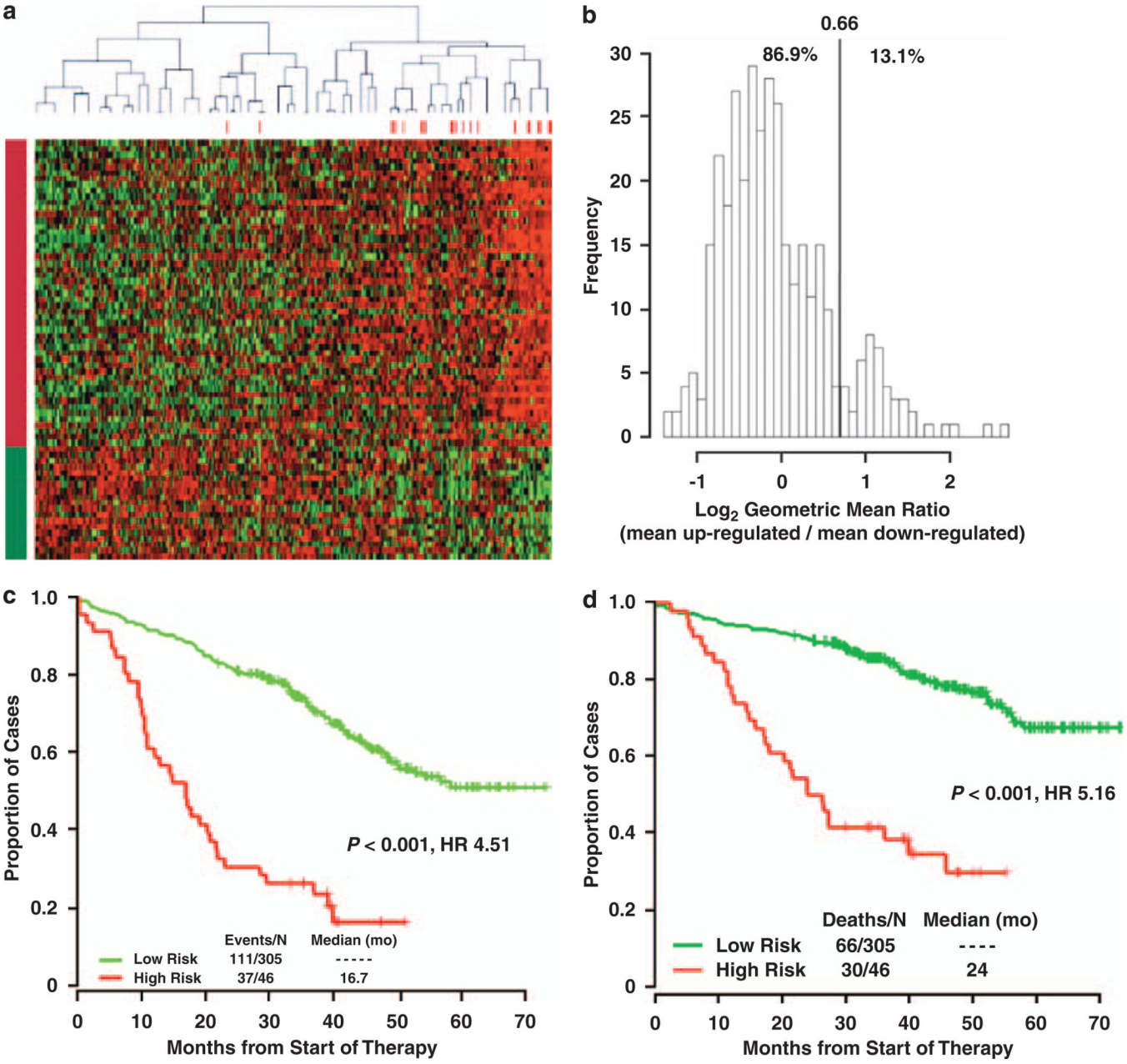

Figure 2.

A GEP-based 70-gene score can define high-risk myeloma. (**a**) Heat map of the 70 genes illustrate remarkably similar expression patterns in $CD138^{+}$ selected tumor cells among 351 newly diagnosed patients. Red bars above the patient columns denote patients with disease-related deaths at the time of analysis. The 51 genes in rows designated by the red bar on the left (top rows; upregulated) identified patients in the upper quartile of expression at high risk for early disease-related death. The 19 gene rows designated by the green bar (downregulated), identified patients in the lower quartile of expression at high risk of early disease-related death. (**b**) Frequencies of the risk score defined as the $\log_2$ geometric mean ratio of the 51 quartile 4 genes and 19 quartile 1 genes. This self-normalizing expression ratio has a marked bimodal distribution, consistent with the upper/lower quartile log-rank differential expression analysis, which was designed to detect genes that define a single

high-risk group (13.1%) with an extreme expression distribution. Interpreted as an up/downregulation ratio on the $\log_2$-scale, higher values are associated with poor outcome. The vertical line shows the high-risk versus low-risk cutoff for the $\log_2$-scale ratio determined by K-means clustering: the percentage of samples below and above the cutoff is also shown. Kaplan–Meier estimates of EFS (**c**) and OS (**d**) in low-risk myeloma (green) and high-risk myeloma (red) showed inferior 5-year actuarial probabilities of EFS (18% versus 60%, $P<0.001$; HR = 4.51) and OS (28% versus 78%, $P<0.001$; HR=5.16) in the 13.1% patients with a high-risk signature. Reproduced with Permission from *Blood*.

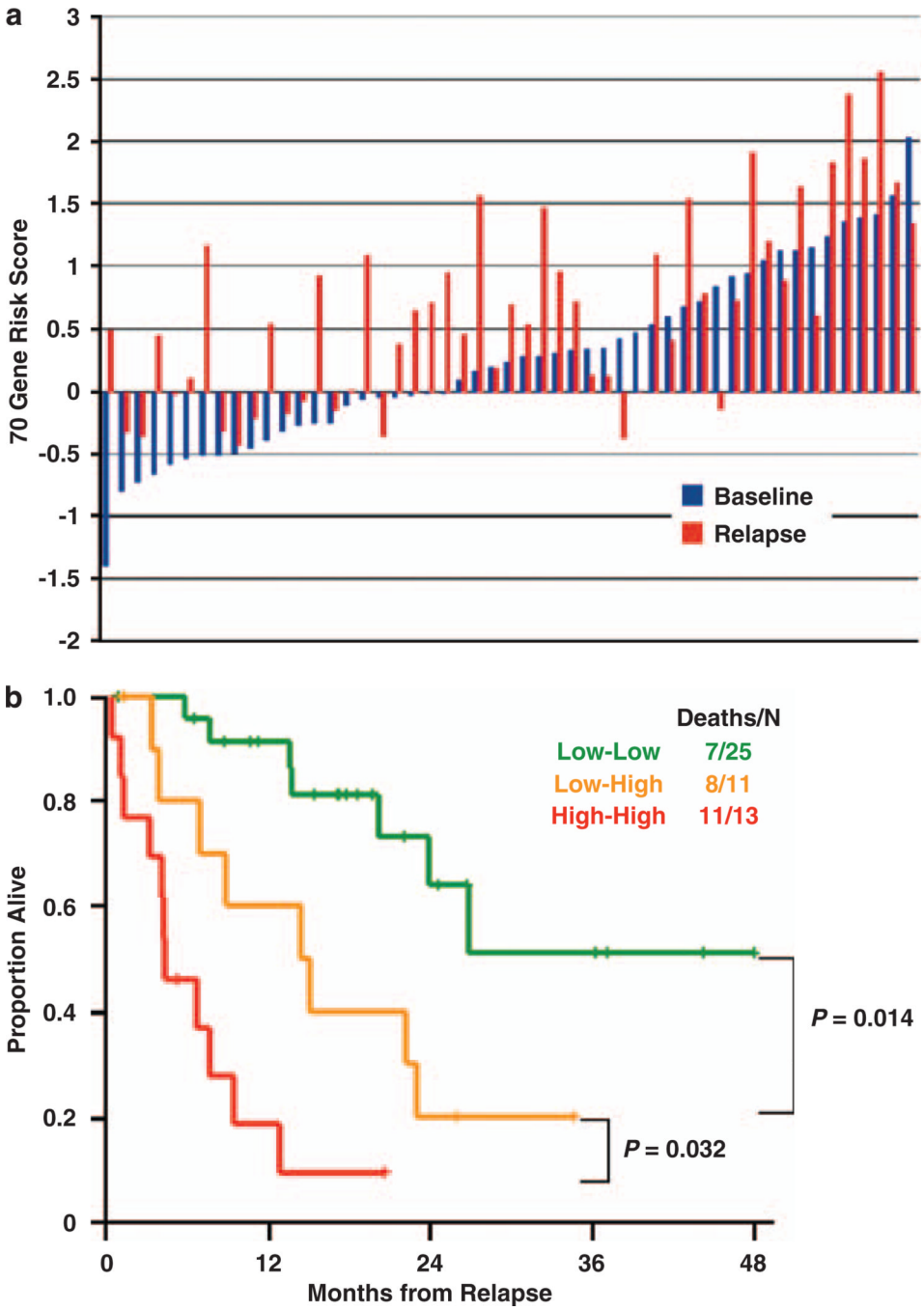

Figure 3.

70-gene risk score can increase in relapsed relative to newly diagnosed disease and an increase predicts poor post-relapse survival. (**a**) The 70-gene risk score in paired diagnostic (blue) and relapse (red) samples of 51 patients. The gene expression risk score is indicated to the left. Sample pairs are order from left to right based on lowest baseline score. (**b**) Kaplan–Meier plot of post-relapse survival of the three groups defined by low risk both at diagnosis and relapse (low-low), low risk at diagnosis and high risk at relapse (low-high), and high risk at both time points (high-high). Reproduced with Permission from *Blood*.

Table 1

Characteristics of validated molecular classes as defined by unsupervised hierarchical clustering

Molecular subtype	%of newly diagnosed patients[a]	Genetic characteristics	Characteristic genes elevated in class	Risk	Features
MS(M**MS**ET)	17	t(4;14)	*FGFR3, MMSET CCND2, IL6R*	High	Overexpression MMSET and FGFR3' FGFR3 not evident in ~30%; bone disease is rare
MF(**M**A**F**/**M**A**F**B)	6	t(14;16) or t(14;20)	*MAF or MAFB CCND2, IL6R*	High/Moderate	Elevated expression of *CCND2*; bone disease rare; low *DKK1*; High NF-kB signature; low TNF-a induced gene *TNFAIP*8.
CD-1 (**C***CN***D**1 or **C***CN***D**3)	6	t(11;14) or t(6;14)	*CCND1 or CCND3*	Low	Few cases express *CCND2* in absence of *CCND1* or *CCND3*; can have high *DKK1*
CD-2 (**C***CN***D**1 or **C***CN***D**3) with CD**2**0 expression	12	t(11;14) or t(6;14)	*CCND1 or CCND3, CD20, VPREB3*	Low	Few cases express *CCND2* in absence of *CCND1* or *CCND3*
HY (**HY**perdiploid)	31	Typical trisomies +3, +5, +7, +9, +11, +15, +19;	*GNG11, DKK1 FRZB*	Moderate	Low-ectopic expression of *CCND1*; del13 and gain of 1q are rare; high expression of interferon-induced genes
LB (**L**ow **B**one disease)	12	Typical HY trisomies; exception is frequent del13, gain of 1q, rare gain of 11	*CCND2, CST6 ARHE, IL6R*	Low	Expression of *CCND2*; low level *DKK1 FRZB,CCR2 HIF1A, SMAD1*; low expression of interferon-induced genes
PR (**PR**oliferation)	10	Made up of all subgroups	*CCNB1, CCNB2, PCNA, MKI67 TOP2A, TYMS*	High	Overexpression of 1q genes; evolves from other groups

[a]Approximately 13% of newly diagnosed cases were not classified. Unclassified samples typically derived from bone-marrow aspirates containing low-bone marrow plasmacytosis. This so-called MY subgroup exhibits superior survival relative to other groups. CD138-purified cells from these cases have GEP of signatures consistent with contamination of preparations with the myeloid, T-cell, B-cell, and plasma-cell lineages. Group consists of all molecular classes described above as spikes and class-specific GEP features are evident in most. Bold and underlined values indicates the source of abbreviations.