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The use of molecular-based risk stratification and pharmacogenomics for outcome prediction and personalized therapeutic management of multiple myeloma

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Abstract

Despite improvement in therapeutic efficacy, multiple myeloma (MM) remains incurable with a median survival of approximately 10 years. Gene-expression profiling (GEP) can be used to elucidate the molecular basis for resistance to chemotherapy through global assessment of molecular alterations that exist at diagnosis, after therapeutic treatment and that evolve during tumor progression. Unique GEP signatures associated with recurrent chromosomal translocations and ploidy changes have defined molecular classes with differing clinical features and outcomes. When compared to other stratification systems the GEP70 test remained a significant predictor of

outcome, reduced the number of patients classified with a poor prognosis, and identified patients at increased risk of relapse despite their standard clinico-pathologic and genetic findings. GEP studies of serial samples showed that risk increases over time, with relapsed disease showing GEP shifts toward a signature of poor outcomes. GEP signatures of myeloma cells after therapy were prognostic for event-free and overall survival and thus may be used to identify novel strategies for overcoming drug resistance. This brief review will focus on the use of GEP of MM to define high-risk myeloma, and elucidate underlying mechanisms that are beginning to change clinical decision-making and inform drug design.

Keywords

Gene-expression profiling; Fluorescence in situ hybridization; Cytogenetics; Multiple myeloma; Classification; Prognosis; High risk; Therapeutic management

1 Introduction

Multiple myeloma (MM) is a malignancy of antibody secreting plasma cells that accumulate within bone marrow. Its sequelae include renal failure, osteolytic bone destruction and osteoporosis, anemia and immunosuppression [1]. Prior to 1999 the primary treatment approach for the MM patient was mainly palliative in nature despite the use of potent chemotherapeutic drugs such as melphalan, prednisone, dexamethasone, vincristine, and adriamycin, as well as stem cell transplantation (SCT) procedures that were introduced in the early 1990s [1–3]. However, the development of novel-acting drugs has become a promising new feature of the therapeutic landscape. The successive introduction of increasingly efficacious drugs such as thalidomide (in 1999), bortezomib (in 2003), and lenalidomide (in 2004) has dramatically improved the clinical outcome for a large proportion of MM patients who can now look forward to substantial increases in progression-free survival (PFS) and overall survival (OS) [4, 5]. Yet, despite this dramatic improvement in therapeutic efficacy, MM remains incurable with a high propensity for eventual relapse in treated patients resulting in a median survival of approximately 10 years [6, 7]. Moreover, a significant portion of newly diagnosed patients (about 15%) do not derive significant benefit from these novel agents either used singly or in combination with stem cell support, and are thus at high risk for early relapse and death [8, 9]. These sobering facts underscore the reality that a number of critical aspects of the biology and underlying genetics of MM in general, and treatment-refractory MM in particular, remain elusive. Consequently, in order to improve clinical outcomes further, a major research focus is to elucidate the molecular and genetic basis for the observed intrinsic and induced resistance to current chemotherapeutic regimens as well as novel targeted approaches to overcome it.

The advent of powerful technologies, such as interphase fluorescence in situ hybridization (FISH), spectral karyotyping, comparative genomic hybridization, single nucleotide polymorphism (SNP) genotyping, and microarray-based gene-expression profiling (GEP), has provided the necessary tools to study MM in exceptional detail. Combining these approaches with increasingly sensitive esoteric technologies, such as high-throughput proteomics, microRNA profiling, whole-genome sequencing, and advances in sophisticated bioinformatic data-mining algorithms, will broaden the defined spectrum of molecular

variables and provide clinicians with relevant therapeutic targets to improve clinical management of the MM patient. GEP, in particular, is a mature technology that can elucidate both the biology and clinical behavior of MM by globally assessing molecular alterations that exist in individual patients at diagnosis and that evolve during tumor progression and after therapeutic treatment. For example, unique gene-expression patterns associated with recurrent chromosomal translocations and ploidy changes have defined molecular classes with differing clinical features and outcome [10]. Furthermore, GEP studies of serial samples showed that risk increases over time, with relapsed disease showing dramatic GEP shifts toward a signature of poor outcomes [11]. This latter observation is significant in that it strongly suggests a common mechanism of disease evolution, driven by a small cohort of genes, and potentially reflects preferential expansion of therapy-resistant cells. Of greater significance, however, is the presumption that correlating GEP-defined disease class and risk with outcomes of therapeutic regimens can reveal specific benefits for individual agents, as well as mechanistic insights into drug sensitivity and resistance. Thus, GEP can play an invaluable role in initial diagnosis by identifying disease subtype, stratifying patients into specific risk groups, and predicting response to chemotherapeutic drugs. As the disease evolves GEP is used to monitor response to therapy, predict clinical outcome on an individual basis, predict disease recurrence, define eligibility for autologous or allogeneic transplantation, and predict response to standard or risk-based therapies. This brief review will specifically focus on the use of GEP of MM to define high-risk myeloma, and elucidate underlying mechanisms that are beginning to change clinical decision-making and inform drug design.

2 Pathogenesis at the molecular level

Several large clinical trials have shown that before any treatment, a high-risk signature is present in approximately 15% of new cases of MM, and is associated with shorter durations of complete remission, event-free survival (EFS), and overall survival [9]. Moreover, even among MM patients diagnosed with low-risk disease who respond to initial treatment, a high percentage of these patients will develop resistance over time and relapse. Therefore, long-term survival in patients with MM can vary considerably, and it is difficult to predict outcome based on current laboratory tests. Present consensus among MM practitioners is that the observed heterogeneity in outcomes for MM patients with similar clinical parameters (e.g., age, clinical presentation, functional status, plasma cell burden, etc.) is the result of a differential evolution of complex genetic abnormalities in both the malignant myeloma cell and the bone marrow microenvironment that collectively promote disease progression. Consequently, a range of genetic and molecular tests have been incorporated as part of the standard of care for MM patients in order to refine diagnosis and prognosis, and to provide more rational clinical guidance regarding selection of a therapeutic modality that would induce the most durable clinical response.

MM is currently characterized either as hyperdiploid containing trisomies, or as non-hyperdiploid containing a number of translocations and other structural rearrangements [12, 13]. The routine use of metaphase cytogenetics and interphase FISH has identified specific recurrent genetic abnormalities that have been associated with inferior outcomes in MM patients and are used as a basis to risk stratify patients [14]. For example, the presence of

several chromosomal abnormalities (CAs), including translocations of the immunoglobulin heavy chain locus on chromosome 14 and chromosomes 4, 16, and 20, deletion of 17p, deletions of all or parts of chromosome 13, and amplification of chromosome 1q have historically been associated with a poor long-term prognosis. Conversely, patients who have extra chromosomes (“hyperdiploidy”), translocation t(11;14), or t(6;14) appear to have longer duration of response to therapy and better survival (i.e., standard-risk myeloma). However, there is broad agreement that this spectrum of genetic abnormalities remains insufficient to capture the significant heterogeneity observed in clinical outcome. Thus, there is a critical need to adopt more precise genetic risk stratification methods at the time of initial diagnosis in order to define and discriminate patients at high risk for early relapse from those at low risk for relapse [15] and to treat patients according to this information.

At the University of Arkansas for Medical Sciences (UAMS), we have pioneered the use of GEP as a highly sensitive method to both stratify MM patients in terms of outcome and to more fully characterize an individual’s tumor at the molecular level. The technique involves extracting RNA from a highly purified population of CD138+ myeloma plasma cells retrieved from the bone marrow and using it to probe a high-density microarray gene chip [16]. Using microarray technology and multivariate analysis of several large cohorts of patients with over a 10-year continuous follow-up and complete clinical and pathological outcomes analysis [9, 17, 18], we identified a distinctive 70-gene molecular signature (GEP70) for high-risk myeloma that correlated with a strong probability of early recurrence and shorter overall survival compared to patients with low-risk MM who had a much greater probability of maintaining a longer-term remission [19, 20]. When compared to other clinically used stratification systems, including the International Staging System (ISS) for multiple myeloma, the GEP70 test remained a significant predictor of outcome (Fig. 1a) [21]. These 70 genes affect overlapping functions known to be important for relapse including cell-cycle regulation, angiogenesis, cell adhesion, cell migration, and proliferation. As compared to standard-risk assessment factors or the presence of abnormal metaphase or interphase genetic abnormalities (Fig. 1b), the GEP70-gene signature significantly reduces the number of patients traditionally classified with a poor prognosis (“false positive”), while at the same time identifying those patients who may be at increased risk of relapse despite their standard clinico-pathologic and genetic findings. Importantly, this test is not only a significant independent variable in multivariate analyses in UAMS datasets but has been validated in other public datasets as well (Table 1).

These analyses have significantly increased our understanding of the molecular heterogeneity of MMs and refined a GEP-based prognostic risk signature that accurately predicts the residual risk of relapsing with active MM in a specific period of time. More importantly, this approach has the ability to predict which patients will be more or less responsive to specific therapeutic modalities, which has enormous clinical implications and sets the stage for a personalized approach to managing the MM patient. However, prior to routine use in the clinic, it is imperative that novel tests like the GEP70 be validated to ensure that it is capable of performing robustly in the varied clinical settings available to the MM patient. The ability of the GEP70 signature to distinguish between high- and low-risk myeloma patients regardless of where or how the MM patient was treated has been validated in other medical centers and in clinical trials using differing clinical treatment protocols

from those utilized at the UAMS as shown by the Phase II CREST and SUMMIT trials [23] as well as the Phase III APEX trials using bortezomib in a relapsed/refractory setting [24]. In addition, the GEP70 signature score has been further validated in a cohort of high-risk patients with specific chromosomal translocations at the Mayo Clinic [25]. Decaux and colleagues 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 better survival [22]. Multivariate analysis comparing the GEP70-gene model with the 15-gene model revealed that the GEP70-gene model was significant in all datasets tested, but the 15-gene model was significant in bortezomib trials only. These data, together with other unpublished data, suggest that the GEP70-gene model captures more outcome variability than models or indices of cell proliferation, considerably improving on currently used stratification metrics.

3 The relationship between GEP and recurrent chromosomal abnormalities

MM is currently classified according to recurrent CAs. An important question, however, is to define the causal relationship of these abnormalities to clinical outcome and if these relationships can be altered by new therapeutic approaches. GEP of large numbers of MM cases has provided sufficient data to divide MM into at least seven definable and reproducible disease classes (Fig. 2) [10]. 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 [26]. These translocations cause normally silent genes to become juxtaposed with powerful immunoglobulin enhancer elements, resulting in expression “spikes” readily detectable in microarray studies (Fig. 2). HY is characterized by a low-level ectopic expression of *CCND1* and overexpression of genes mapping to the odd-numbered chromosomes that typically exhibit trisomy in MM. The LB 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*. The seventh class of MM, PR, 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 allow segregation of these seven molecular MM subclasses [10].

The MS class of MM, which is characterized by the t(4;14)(p16;q32) translocation, is a high-risk subtype that predicts poor prognosis [27]. The t(4;14)(p16;q32) reciprocal translocation results in hyperactivation of both *FGFR3* and *MMSET/WHSC1* genes [28]. All t(4;14)-positive disease expresses elevated levels of *MMSET*, but in about 30% of these cases expression of *FGFR3* is lost [27, 29]. Because loss of *FGFR3* expression is the only obvious GEP difference between these two types of t(4;14)-positive MM, it appears that *MMSET* plays a central role 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 [30]. Functional studies suggest that *MMSET* plays a role in cell proliferation by decreasing cell viability and cell-cycle progression.

Accounting for approximately 6% of cases, the 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 also 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 less than 5% of MM cases, *c-MAF* expression is elevated in myeloma cell lines lacking the translocations and in up to 50% of primary samples [31]. 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. 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 [32]. 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.

The t(11;14)(q13;q32) and t(6;14)(p21;q32) translocations, characteristic 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, non-overlapping 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 [10], but cells expressing elevated *PAX5* mRNA do not express the protein [33]. 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 [10].

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 previously shown to have a distinct gene-expression signature [34]. The HY signature is present in nearly 50% of cases and is associated with hyperdiploid karyotypes in more than 90% of these; however, the signature is also observed in cases that are not identified as hyperdiploid by flow cytometry analyses.

A novel class of MM, LB (low bone disease), 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 (PET) imaging with GEP also revealed that the LB class is uniquely inversely correlated with F18-fluorodeoxyglucose PET-defined focal lesion number and intensity [35]. 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 via suppression of Wnt/ β -catenin signaling antagonist *DKK1* [36, 37], and the LB class is characterized by significantly lower expression of *DKK1*, suggesting that EDN1 may downregulate *DKK1* in myeloma cells.

The PR (proliferation) 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 non-hyperdiploid cases are equally common, with and without concomitant translocation spikes, suggesting that hyperdiploid versus non-hyperdiploid status likely is 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 upon disease progression—suggest that the PR class is driven by a transformation event secondary to underlying primary genetic lesions.

These molecular classes are largely consistent with the TC classification schema of Bergsagel et al. [34] who used correlated gene-expression profiles with specific recurrent translocations, trisomies, and expression of cyclin D genes to identify eight translocation/cyclin D (TC) groups. These were termed the 11q13/TC1, 6p21/TC2, 4p16/TC3, maf/TC4, D1/TC5, D1+D2/TC6, D2/TC7, and none/TC8 classes [38]. 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 [38]. Genomic profiling of a large number of MM cases 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* [38]. *IGH*-mediated translocations can directly activate *CCND1* (11q13) [39] or *CCND3* (6p21) [40]; *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 [31]. Biallelic dysregulation of *CCND1* occurs in nearly 40% of tumors, most of which are hyperdiploid [38]. 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 above) [10].

Of significant note is that the GEP70 high-risk signature is evident in a subset of all MM molecular classes defined to date. Moreover, the presence of this high-risk signature even in MMs defined as low-risk by conventional cytogenetic and FISH analysis, negatively

influences clinical outcome in these patients: e.g., low-risk MS subclass disease fares much better than high-risk MS disease.

In addition to its ability to predict the outcome of newly diagnosed MM patients, the GEP70-gene model is also an independent and significant prognostic factor in predicting post-relapse survival (PRS) in relapsing MM [11]. We assessed 120 MM patients previously enrolled in tandem transplantation trial Total Therapy 2 and observed that the 3-year PRS was 71% in patients with GEP70 low-risk disease present both at baseline and relapse (Fig. 3a) [11]. This contrasted with only 17% of patients with GEP70 high-risk disease at relapse. On multivariate analysis of relapse parameters, we showed that a GEP70 high-risk signature conferred short PRS. Thus, the conversion from GEP70 low- to high-risk severely impacted PRS and was unrelated to the salvage therapies used. Recognizing that GEP risk status increases in a sizeable proportion of patients, GEP analysis can be repeated on treatment failure so that salvage strategies can be selected according to this powerful risk-discriminator. Improving on risk stratification provided by the ISS, Mulligan and colleagues 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 [23]; a modified version of the GEP70-gene model also predicted poor outcome in relapsed disease [24].

The marked increase in the frequency of a GEP70 high-risk designation from 13% at diagnosis to 76% at relapse provides strong molecular evidence of disease evolution that influences post-relapse outcome, indicating that the underlying molecular mechanisms that drive disease progression can be identified. We observed that this quantifiable increase in the high-risk score over time was concordant with gains of chromosome 1q [19], suggesting that expansion of a dominant clone with survival and/or proliferation advantages may involve altered regulation of genes on this chromosome. Abnormalities of chromosome 1 are among the most frequent chromosomal alterations in MM being observed in >45% of patients [41] with the short arm being often associated with deletions and the long arm with amplifications [42]. Gains/amplification of 1q21 increases the risk of MM progression and incidence of the amplification is higher in relapsed than in newly diagnosed MM [41, 43]. GEP studies comparing MM with and without 1q gains [44] 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. Integrating GEP and DNA copy number variation data, several independent studies revealed that numerous 1q genes are copy number sensitive in MM [42, 45]. Taken together these data show that there is a correlation between cytogenetic abnormalities identified by FISH analysis and GEP data in MM (Table 2).

Gains of 1q are associated with upregulation of genes involved in intracellular protein transport; including *COPA* and *ARF1*, which play roles in vesicle-mediated transport from the endoplasmic reticulum (ER) to the Golgi, and *RAB1F* 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. In cases with 1q gains, three genes involved in protein translation (*RPLP2*, *RPL21*, and *FAU*) are downregulated, 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 [46]. Cases with 1q gains also showed significantly modulated expression of genes involved in ER stress-induced responses, including upregulation of *CLN3* (chaperone), *UBAP2L* and *UBE2Q1* (ubiquitin cycle), *PSMD4* (proteasome degradation), and *CASP4* (initiates apoptosis in response to ER stress) [44]. The importance of the unfolded protein response (UPR) in maintaining tissue health is evidenced by the fact that a chronic increase in defective protein structures plays a pathogenic role in diabetes, cardiovascular disease, Alzheimer’s and Parkinson’s syndromes, and cancer. Since the UPR promotes cell survival by reducing the accumulation of aberrantly folded proteins through translation arrest, production of chaperone proteins, and increased degradation, and because ER stress-induced apoptosis can play an important role in malignant cells’ sensitivity to certain drugs, including bortezomib, a better understanding of ER stress-induced responses may contribute to important new treatment strategies [44].

4 Risk factors in the era of novel agents based on GEP treatment options

In the new era of targeted therapies and novel drugs, the clinical focus has rapidly shifted from treating MM patients as a monochromatic biological group to one where the ultimate goal is to predict which drug regimen will provide the optimal outcome for an individual patient. The ability to assess the genetic response of a particular MM to an administered drug will be of enormous advantage in selecting the most efficacious approach. However, since MM is not a single genetically distinct disease, but one with many molecular subtypes that manifest significant differences in clinical presentation and outcome, sensitive techniques like GEP that identify specific defects which accurately predict treatment options for newly diagnosed and relapsed/refractory MM patients are critical. This is of particular importance in the era of novel therapies since the introduction of agents like bortezomib and lenalidomide have reduced the prognostic relevance of currently used high-risk CAs identified by FISH and cytogenetics such as t(14;16) and t(4;14), either because these new drugs overcome the molecular dysfunction caused by these genetic abnormalities or because they are not primary drivers of disease progression. For example, although many studies predict a poor prognosis for patients who manifest t(4;14) translocations [25, 47, 48], recent studies at the University of Arkansas have shown a large proportion of these high-risk patients who undergo long-term treatment with bortezomib can on average expect almost similar outcomes as patients with standard-risk myeloma [49]. Other studies have confirmed these data [50, 51]. However, despite intensive use of the current arsenal of drugs as well as incorporation of stem cell support, there continues to be a subset of ultra-high-risk MM patients that have inferior outcomes indicating the impact of other genetic abnormalities that continue to exert a dominant role in tumor progression and which are unaffected by current therapeutic approaches. For example, the presence of 17p13 deletions still predicts an inferior outcome even with novel drug therapy [13]. This high-risk feature presumably leads to loss of heterozygosity of *TP53*, a tumor suppressor gene that transcriptionally regulates cell-cycle progression and apoptosis to modulate cellular responses to DNA damage. Xiong

et al. [52] 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 more than 122 known p53 target genes were highly correlated with *TP53* expression in primary myeloma cells. GEP following 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 [52].

MicroRNAs (miRNAs) are noncoding RNAs that regulate global gene expression. The miRNAs often act synergistically to repress target genes, and their dysregulation can contribute to the initiation and progression of a variety of cancers. To investigate the potential involvement of miRNA in MM, we used whole-genome microarray analyses of CD138-enriched plasma cells from 52 newly diagnosed cases of MM to correlate miRNA expression profiles with the GEP70 risk score, proliferation index, and predefined gene sets [53]. In stark contrast to mRNAs, we discovered that all tested miRNAs were significantly upregulated in high-risk disease as defined by GEP70 risk score and proliferation index. Increased expression of *EIF2C2/AGO2*, a master regulator of the maturation and function of miRNAs and a component of the GEP70 risk model, is driven by DNA copy number gains in MM [53]. Genome-wide elevated expression of miRNAs in high-risk MM may be secondary to deregulation of AGO2 and the enzyme complexes that regulate miRNA maturation and function. Thus, additional detailed molecular studies will no doubt further clarify which genetic/CAs are pivotal to the various dysregulated biological attributes of MM and which are susceptible to modulation by current drug regimens.

5 Using GEP to predict therapeutic response

The ability to generate detailed knowledge of the molecular defects in a cancer holds significant potential to identify which drug or combination of drugs will impact that specific cancer and provide long-term benefit to an individual patient. 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. For example, as a way to determine which genes may confer drug resistance, GEP has been used to identify the set of genes impacted in MM cells after exposure to two clinically relevant drugs, dexamethasone and bortezomib in vitro [54–56]. Similar studies have used GEP to identify the transcriptional signatures of a number of potentially active drugs in MM cells, such as 2-methoxyestradiol (2ME2), an estrogen derivative [57], two histone deacetylase inhibitors, SAHA and VPA [58, 59], insulin-like growth factor receptor (IGFR) inhibitors [60], a IL-6 receptor inhibitor, Sant7 [61], an inhibitor of Wnt signaling pathway, PKF115-584 [62], ribonucleotide reductase inhibitor, Didox [63], and Atiprimod, an oral agent with anti-inflammatory properties [64]. Using GEP, the transcriptional signatures of cellular responses

to 5-aza-2'-deoxycytidine and trichostatin [65], a HSP90 inhibitor, 17-AAG [66], and a marine-derived compound, Zalypsis [67] have also recently been described.

An excellent example of the power of GEP to study drug responses of MM patients in vivo was provided by Burington et al. who compared GEP signatures of myeloma cells before and 48 h after single-agent therapy with dexamethasone, thalidomide, or lenalidomide. The results showed that genes differentially expressed after therapy were prognostic for EFS and overall survival (OS) [68]. 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 [68]. 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 disease.

A second study assessed GEP signature differences following therapy with the proteasome inhibitor bortezomib in 142 newly diagnosed symptomatic MM cases and identified 80 highly survival-discriminatory genes [69]. Analysis of the 80 genes revealed two major gene clusters with the upper gene cluster comprising many genes coding for subunits of the proteasome. The 26 patients in the high-risk groups demonstrated a concerted upregulation of 42 "bad" genes (including genes coding for the proteasome) and downregulation of 38 "good" genes. In the context of the 70-gene risk model, the GEP80 model identified 9% of patients with a grave prognosis among those with GEP70-defined low-risk disease and 41% of patients with a favorable prognosis among those with GEP70-defined high-risk disease (Fig. 3b) [69]. *PSMD4*, which is common to both models, resides on chromosome 1q21 and is associated with progressively shortened PFS and OS with an increase in copy number [69]. GEP80 baseline-defined high risk, high LDH, and low albumin were the only independent adverse variables surviving the multivariate survival model. 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 [69].

Finally, a recent study by Rajkumar and colleagues [70] examined the utility of GEP-based risk stratifications systems in newly diagnosed patients undergoing therapy with lenalidomide and dexamethasone in the context of a Phase III trial. Among the major findings from this study were that the high-risk GEP70 signature had excellent prognostic value in predicting inferior OS in the context of primary treatment with novel agents alone. In addition, a GEP70 high-risk signature portends a poorer outcome in patients treated primarily with novel agents like lenalidomide.

6 Conclusions

Utilizing high-throughput genomic analyses and datamining 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 following 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. Thus, GEP is emerging from the research laboratory as a clinical tool with the potential for transforming routine management of MM. While the majority of MM patients can anticipate long-term disease control via 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 [15]. Armed with such a stratification algorithm, it is anticipated that physicians will have the ability to individualize treatment options, improve therapeutic efficacy and clinical outcomes, minimize adverse effects, perform fewer diagnostic tests, decrease unnecessary treatments, and reduce the clinical and financial burden to health care systems and individual patients. For patients with MM, the availability of accurate stratification tools like the GEP70 constitutes a major inflection point in the clinical management of the MM patient that could have an important impact on overall survival and quality of life. Genetic profiling by various means will eventually uncover sufficient genetic understanding that one or more targeted therapies will produce not only long time remissions but hopefully true cures.

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Fig. 1.

Impact of gene-expression profiling (GEP)-defined risk across Total Therapy (TT) regimens in newly diagnosed patients **a** within International Staging System (ISS) stage subsets on overall survival in ISS-defined low risk (Stage I, *left*), intermediate risk (Stage II, *center*) and high risk (Stage III, *right*) or **b** with cytogenetic abnormalities (*left*), with low *TP53* (indicative of deletion [52], *center*) or that harbor *t(4;14)* (*right*). Adapted from [21]

Fig. 2.

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. 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 (*lower panel*). 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. Adapted from Zhan et al. [10]

Fig. 3.

Impact of the GEP70 risk model. **a** Post-relapse survival (PRS) by GEP-defined risk in the context of GEP data at both baseline before therapy and at relapse revealed that PRS was superior in the absence of high risk at both observation times. **b** Applying the GEP80 post-bortezomib (PB) model to GEP70-defined low- and high-risk disease revealed significant segregation in survival outcomes. OS in both GEP70 low- and high-risk settings could be further discriminated by the GEP80(PB) model. Best outcomes were observed in patients with GEP70 low-risk and GEP80(PB) low-risk (LL) features, followed by those with GEP70 high-risk and GEP80(PB) low-risk (HL) features and those with GEP70 low-risk and GEP80(PB) high-risk (LH) features; worst outcomes were observed in patients with GEP70 high-risk and GEP80(PB) high-risk (HH) characteristics. The LH group exhibited poorer survival than the HL group, however, there are no significant differences between the two groups (p value .268). Adapted from [11, 69]

Table 1

Studies incorporating multiple myeloma GEP70 risk model in outcome prediction

Study	Type of treatment	Patient (n)	GEP70 independence in MVA	Tests completed	% population high risk	Conclusions	References
Comparison of ISS, CAs and GEP risk stratification	TT2: induction, Mel-based ASCT, consolidation, maintenance TT3: added Bzb	Newly diagnosed (TT2, 351) (TT3, 275)	Yes	ISS3 CA GEP	21 33 14	After accounting for GEP-derived risk and delTP53 status, ISS stage, and CAs still retain independent prognostic implications. GEP high risk is identified as having the highest HR for death and EFS. The GEP70 model identifies high-risk patients with shorter OS in each of the ISS stages	[21]
GEP of relapsed myeloma	TT2	Relapsed (120)	Yes	CA GEP	68 38	GEP-defined high-risk status at relapse predicts shorter OS, while GEP-derived HY subgroup at relapse implied a decreased risk for death in MVA	[11]
GEP-defined proliferation for prognosis	HM1 and HM2: Mel, ASCT	Newly diagnosed (298)	ND	GEP-PI ISS3 GEP15 GEP70	7.7 17 12.4 18.7	Proliferation of malignant plasma cells, assessable by GEP-defined proliferation indices, is a central biological and prognostic factor in MM, independent of B2M, ISS-stage, high risk-associated CAs, and gene expression-based risk scores [71]	
GEP-defined PI and risk: t(4;14), low TP53	Mayo Clinic: high-dose Mel, ASCT	Newly diagnosed (71)	ND	GEP17 ^a	13	GEP 17 identifies a high-risk subgroup with shorter OS in the Mayo Clinic dataset [25]	
GEP-defined risk in MM patients receiving Len and Dex	E4A03: Len plus Dex or Dex	Newly diagnosed (45)	ND	CA ISS3 GEP15 GEP70	23 21 22 16	GEP70 and GEP15 identify a high-risk subgroup with shorter OS in the non-autotransplant population thus highlighting the importance of GEP scoring in the setting of initial therapy with Len and Dex [70]	
Identify broadly applicable prognostic markers	IFM 99: double ASCT, then no treatment or Pam or Thal w/Pam	Newly diagnosed (250)	Yes	ISS3 GEP15 GEP17	22.5 25 25	GEP 17 independently identifies high-risk myeloma in 5 independent datasets. GEP17 outperforms GEP15 (IFM-model) when tested on the Dex only arm of the APEX trial. GEP17 identifies high-risk myeloma in the IFM dataset. GEP15 does not remain an independent significant variable in the UAMS dataset [22]	
Phase III APEX trial	Bzb or Dex, Dex pts w/ progressive disease receive Bzb	Relapsed, 1–3 or >3 lines of therapy (156)	ND	GEP17	13.5	GEP17 identifies a high-risk subgroup in the APEX dataset that has a significantly shorter OS [24]	
Homozygous deletion mapping identifies genes and an associated GEP in MM	MRC Myeloma IX: Younger: ASCT, Cyc, Thal and Dex or Cyc, Vin, Adr and Dex. Older: Cyc, Thal, and Dex or Mel and prednisolone	Newly diagnosed (258)	Yes	GEP97 GEP15 GEP70	34 25 35	The Cell death-97 GEP identifies 89 cases with poor prognosis, and the IFM 15-GEP identifies 64 cases. The GEP97 is almost equally as sensitive to identifying poor-prognosis cases as the GEP70, which identifies 90 cases [72]	

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Gene-expression profiling (GEP), multivariate analysis (MVA), proliferation index (PI), autologous stem cell transplant (ASCT), cytogenetic abnormalities (CA), international staging system (ISS), beta-2-microglobulin (B2M)

Bz/b bortezomib[®], *Thal* thalidomide, *Dex* dexamethasone, *Mel* melphalan, *Cyc* cyclophosphamide, *Vin* vincristine, *Adr* adriamycin, *Pam* pamidronate, *Len* lenalidomide

^a GEP17 was derived from, and can substitute for, GEP70 [19]

Table 2

Studies correlating data obtained from FISH with data obtained from GEP

Cytogenetic Abnormality	Conclusion	References
t(6;14)	By meta-phase chromosome analysis, the t(6;14)(p21;q32) translocation was identified in 6 of 150 (4%) primary MM tumors. Overexpression of cyclin D3 messenger RNA (mRNA) was identified by microarray RNA expression analysis in 3 of 53 additional primary MM tumors, each of which was found to have a t(6;14) translocation breakpoint by interphase fluorescence in situ hybridization analysis (FISH)	[40] [73] [74]
t(4;14)	t(4;14) is detected by FISH in 18% of newly diagnosed MM cases and is strongly associated with over expression of MMSET. 32% of these cases lack expression of FGFR3	[29] [73] [74]
t(11;14)	11 of 11 MM samples with spiked expression of CCND1 by GEP had a detectable t(11;14) by TRI-FISH	[16] [73] [74]
t(14;16)	Gene-expression profiling analysis revealed strong peaks of MAF expression in the cases with t(14;16) detected by FISH	[73]
t(14;20)	c-MAF, which is deregulated by t(14;16), and MAFB, deregulated by t(14;20), were observed only in a subset of patients, which clustered separately within this MF cluster. The remaining samples in the MF cluster clustered with these samples on the basis of overexpression of downstream targets of MAFB and/or c-MAF: RND3, CCND2, and ITGB7	[74]
del 13	Ten genes, including RB1, were identified as correctly discriminating 41 (94%) of 48 cases from validation group in which FISH 13 status was known	[75]
del 17p13	TP53 deletion was highly correlated with low TP53 expression. Comparison of TP53 expression level on the basis of deletion status revealed that TP53 expression was lower in 36 monoallelic deletion cases and even lower in 4 biallelic deletion cases than in 154 cases lacking deletion	[52] [74]
amp 1q21	Interphase fluorescence in situ hybridization revealed that CKS1B expression was strongly correlated with DNA copy number in a subset of 197 cases with both measurements by GEP and FISH for 1q21	[44] [76]