Multiple Myeloma: Is It Time for Biomarker-Driven Therapy?
Manisha Bhutani, MD, Ola Landgren, MD, PhD, and Saad Z. Usmani, MD, FACP

OVERVIEW

Remarkable strides have been made in understanding the molecular mechanisms by which multiple myeloma develops, leading to more sophisticated classification that incorporates not only the traditional diagnostic criteria, but also immunophenotype, genetic, and molecular features. However, even with this added information, considerable heterogeneity in clinical outcomes exists within the identified subtypes. The present paradigm for myeloma treatment is built on the basic step of defining transplant eligibility versus noneligibility, as determined by age, performance status, and cumulative burden of comorbidities. An incredibly complex heterogeneous disease is, therefore, treated in a generalized way with the result that large interpatient variability exists in the outcome. As antmyeloma therapeutics continue to expand it is becoming even more crucial to personalize treatment approaches that provide the most value to a specific patient. Development of biomarkers, either individually or as larger sets or patterns and ranging from analysis of blood or bone marrow to biomedical imaging, is a major focus in the field. Biomarkers such as involved serum free light chain ratio and MRI focal lesions have been implemented in the new definition of multiple myeloma and guide clinicians to initiate treatment in otherwise asymptomatic individuals. Currently, however, there is not enough evidence to support intensifying the treatment for high-risk disease or reducing the treatment for low-risk disease. Minimal residual disease-negative status is an important biomarker that holds promise for monitoring the effectiveness of response-adapted strategies. This article sheds light on the forward landscape and rear-mirror view of biomarkers in myeloma.

The outcome for patients with multiple myeloma (MM) has considerably improved over the last decade with the incorporation of active agents including immunomodulatory drugs (IMiDs) and proteasome inhibitors, such that the median survival of newly diagnosed patients is now between 5 and 9 years. Increasingly effective salvage therapies have resulted in durable remission as well as long-term survival for selected patient subgroups. Nonetheless, despite these advances the current therapy for MM remains noncurative, and a subset of cases show a rapidly relapsing and refractory disease course. Myeloma therapy is currently based on studies that largely predate the molecular classification, and most patients are treated by the conventional one-size-fits-all therapeutic approach. Over recent years, great progress has been made in our understanding of the key genetic abnormalities, central signaling pathways, the role of the bone marrow microenvironment, and monitoring of minimal residual disease (MRD). It is undoubtedly clear that there is extensive heterogeneity, not only between patients but also within patients, resulting from complex genetics and clonal evolution during the course of disease. The MM genome is extremely complex, with approximately 35 nonsynonymous mutations identified per case. Underlying this vast landscape of genetic alterations are multiple deregulated core signaling pathways and mutations of diagnostic and therapeutic significance that open several possible avenues for the optimization of risk-adapted strategies and biomarker-focused clinical trials in MM. A biomarker is typically defined as any characteristic (e.g., gene, protein, clinicopathologic variable, imaging feature) that can be objectively and reproducibly measured to serve as an indicator of disease biology or response to a therapeutic intervention. For clinical purposes, disease-related biomarkers assist in diagnosis, prognosis, and response monitoring. Drug-related biomarkers, on the other hand, indicate whether a drug will be effective in a specific patient and how the patient’s body will process it. Within the context of a clinical trial, an integral biomarker is defined as a marker that must be measured in real time for the trial to proceed, for example when the test is used to establish eligibility, treatment assignment, or stratification, or to detect early response to decide further treatment. In contrast, integrated biomarkers are measured during the clinical trial; however, their results do not determine the treatment or course of the ongoing trial, but instead inform future studies. Biomarker research in myeloma continues to advance in many spheres. The first pertains to biomarkers that aid in risk stratification of patients and provide guidance in the selection or titration
of therapeutic agents. Second, it is becoming increasingly apparent that certain biomarkers offer the promise of distinguishing aggressive from indolent disease evolution, such that individuals most at risk of progressing from precursor states to MM can be targeted at the earliest and most treatable stage. The third area of thrust is biomarkers of benefit, which indicate the effect of therapeutic intervention and predict sensitivity or resistance, and thus correlate with outcomes. Additionally, the technologies for standardization and interpretation of MRD continue to evolve. Clinical trials based on integral and integrated biomarkers remain a key area of research, and correlative science within the context of these trials will be vital in determining the significance of defined biomarkers. We provide an overview of the current knowledge in each area in which biomarkers are being explored in MM. In addition, we discuss the potential and limitations of designing biomarker-driven clinical trials.

MONOCLONAL PROTEIN BIOMARKERS

Myeloma-related plasma cell disorders are typically characterized by monoclonal protein biomarkers, which can be in the form of intact immunoglobulin, immunoglobulin fragments, or free immunoglobulin light chains (FLC), in either the serum or urine. These biomarkers play an important role in diagnosis and response monitoring. One of the earliest identified biomarkers is Bence Jones protein, which was described in 1848. A cutoff of serum monoclonal protein of 3 g/dL or greater and/or bone marrow plasmacytosis of at least 10% is used to distinguish smoldering multiple myeloma (SMM) from monoclonal gammopathy of undetermined significance (MGUS). During the past decade the measurement of serum kappa and lambda FLC has also become part of routine clinical testing. An abnormal FLC ratio indicates the presence of clonality in approximately one-third of patients with MGUS and in at least 90% of patients with MM. The assay is particularly indicated for the diagnosis and follow-up of patients with nonsecretory or oligosecretory myeloma, light chain myeloma, and amyloidosis. Revised International Myeloma Working Group (IMWG) 2014 criteria define myeloma biomarkers that indicate a requirement for therapy in asymptomatic individuals, including bone marrow plasmacytosis of 60% or greater and involved FLC ratio greater than 100 (Table 1). In an era of broadened treatment options, there are substantial data showing the association of depth of response and outcome. Following treatment, complete response (CR) criteria include negative immunofixation of serum and urine and the presence of less than 5% plasma cells. Normalization of the FLC ratio plus the absence of clonal plasma cells by immunohistochemistry or immunofluorescence is considered a deeper level of response that is termed stringent CR (sCR). Achievement of CR is considered one of the strongest prognostic biomarkers in MM, both in the transplant and nontransplant settings, although the sCR criteria have failed to unequivocally demonstrate superior prognostic value compared with CR. More sensitive ways of measuring CR are required when results from serum protein electrophoresis are difficult to interpret, including problems resulting from polyclonal immunoglobulins, comigration of monoclonal bands, and poor sensitivity at low levels of monoclonal protein (<10 g/L). These drawbacks could be overcome by the new U.S. Food and Drug Administration (FDA)-approved heavy/light chain (HLC) assay (Hevylite). The unique ability of this assay to measure suppression of the uninvolved HLC pair (e.g., IgG-lambda, IgA-kappa, and IgA-lambda for a patient with IgG-kappa disease) adds sensitivity for monitoring disease response and detecting residual disease. The HLC ratio reflects the balance between monoclonal and polyclonal immunoglobulins of involved and uninvolved isotypes taking into account the polyclonal plasma cell suppression or expansion that occurs with the treatment. A few studies have shown that this assay affords additional prognostic information in MGUS and MM. If confirmed by other studies and long-term follow-up, HLC could be a noninvasive marker of response.

CYTOGENETICS AND FISH BIOMARKERS

Many studies have reported on the prognostic value of cytogenetics and fluorescence in situ hybridization (FISH) biomarkers; however, some have yielded conflicting results. Reasons for these discrepancies include retrospective analyses, small sample size, variable techniques, lack of a standard definition of high risk, and failure to control for other biologic or treatment processes that may confound the outcome. In general, t(14;16), t(14;20), and del(17p) are associated with a poor prognosis, whereas t(11;14), t(6;14), and hyperdiploid myeloma are considered to impart standard risk, provided there are no additional adverse features such as +1q21 and del(17p) (Table 1). Trisomies are typically associated with a
### TABLE 1. The Changing Biomarker Landscape

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Old</th>
<th>Updated</th>
<th>Future</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Smoldering Multiple Myeloma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diagnostic</strong></td>
<td>≥ 10% clonal marrow plasma cells*</td>
<td>10% to 60% clonal marrow plasma cells</td>
<td>High circulating clonal plasma cells by flow cytometry</td>
</tr>
<tr>
<td></td>
<td>≥ 3 g/dL serum M-protein</td>
<td>Urinary M-protein ≥ 500 mg per 24 h</td>
<td>10% increase in M-protein within 6 months</td>
</tr>
<tr>
<td><strong>Prognostic</strong></td>
<td>Serum free light-chain ratio 0.125 to 8; immunoparesis; ≥ 95% marrow plasma cells of aberrant phenotype** by flow cytometry</td>
<td>High bone marrow plasma cell proliferative rate; GEP 70 Risk score ≥ 0.26; Cytogenetic subtypes: t(4;14), 1q amp, or del 17p</td>
<td></td>
</tr>
</tbody>
</table>

| **Multiple Myeloma** | | | |
| **Diagnostic** | Clonal bone marrow plasma cells ≥ 10% or biopsy-proven bony or extramedullary | Clonal marrow plasma cell ≥ 60% |
| | Plasmacytoma PLUS | Involved/uninvolved serum free light chain ratio ≥ 100 |
| | CRAB myeloma defining events | > 1 focal lesion on MRI§ |
| | | CRAB events including bone lesions on CT or PET/CT |
| **Low/Standard Risk†** | Trisomies | ISS I/II and NO t(4;14) or del 17p3 | New GEP-based prognostic signatures |
| | Hyperdiploidy | Age < 55 |
| | ISS I | t(11;14) |
| | ISS II | t(4;14) |
| | | 1q gain |
| | | Deletion 13 or hypodiploidy by conventional karyotyping |
| | | Complex karyotype |
| | | Plasma cell labeling index > 3% |
| **Standard/Intermediate Risk** | ISS III | ISS I/II plus t(4;14) or del 17p3 |
| | Monosomy 13 | t(4;16) |
| | Hypodiploidy | Plasma cell leukemia |
| | t(4;14) | LDH ≥ 2 ULN |
| **High Risk** | ISS III | ISS I/II plus t(4;14) or del 17p3 |
| | Monosomy 13 | t(4;16) |
| | Hypodiploidy | Plasma cell leukemia |
| | t(4;14) | LDH ≥ 2 ULN |

| **Treatment/Response** | CR | sCR | MRD-negative |
| | | Immunophenotypic CR | HLC ratio |
| | | | Immune signature |
| | | | Proteasome signature |
| | | | IMiD signature |
| | | | Clone burden/Clone status |

| **Imaging Prognostic** | Skeletal survey lytic lesions | MRI focal lesions > 7 | Novel PET probes |
| | FDG/PET focal lesions > 3 | DCE-MRI diffusion/perfusion |

**Abbreviations:** LDH, lactate dehydrogenase; ULN, upper limit of normal; sCR, stringent CR; GEP, gene expression profiling; MRD, minimal residual disease; IMiD, immunomodulatory drugs; DCE-MRI, dynamic contrast enhanced-magnetic resonance imaging.

*Clonality should be established by the demonstration of κ/λ-light-chain restriction on flow cytometry, immunohistochemistry, or immunofluorescence. Bone marrow plasma cell percentage should preferably be estimated from a core biopsy specimen; in cases of disparity between the aspirate and core biopsy, the highest value should be used. M-protein: monoclonal protein.

**Aberrant immunophenotype of plasma cells on flow cytometry: absence of CD19 and/or CD45 expression; overexpression of CD56, or weak expression of CD38. CRAB: hypercalcemia, renal insufficiency, anemia, and bone lesions.

†Mayo Clinic risk classification includes standard risk, intermediate risk, and high-risk categories; International Myeloma Working Group consensus risk classification includes low risk, standard risk, and high-risk categories. ISS: International Staging System: stage I: serum beta-2 microglobulin < 3.5 mg/dL, albumin ≥ 3.5 g/dL; stage II: beta-2 microglobulin between 3.5 and 5.5, or albumin < 3.5 g/dL; stage III: beta-2 microglobulin ≥ 5 mg/dL. CR: complete response.

‡These values are based on the serum Freelite assay (The Binding Site Group, Birmingham, UK). The involved free light chain must be ≥ 100 mg/L.

§Each focal lesion must be at least 5 mm in size. PET-CT: 18F-fluorodeoxyglucose PET with CT.
better prognosis, except when associated with gains of 1q and monosomy of chromosome 13. Gain of 1q is seen in approximately 40% of newly diagnosed cases, and has been linked to adverse prognosis in patients treated intensively with or without IMiDs.23,24 Although monosomic 13/13q deletion (present in approximately 50% of cases) has been typically associated with an unfavorable prognosis when detected by conventional cytogenetics,25 the prognostic implication of this abnormality is difficult to assess because of its close association with other high-risk genetic features, such as t(4;14), in 80% of cases.19 Approximately 32% of patients presenting with MM have a deletion of 1p affecting FAFl and CDKN2C, which has been associated with short survival.26 Some of these biomarkers have treatment-specific prognostic implications; for example, an approach that combines bortezomib-based therapy for 2 to 3 years in conjunction with tandem autologous stem cell transplantation (ASCT) has been shown to abrogate or attenuate the prognostic influence of t(4;14).27,28 No single genetic abnormality by itself defines high-risk MM and it is important to determine the presence or absence of a panel of cytogenetic abnormalities to properly identify patients with adverse prognosis.

INTERNATIONAL STAGING SYSTEM
The International Staging System (ISS) was developed as a multicenter effort before IMiDs and proteasome inhibitors became available and has been the primary clinical tool used to predict outcome.29 This system stratifies patients into three groups based on serum albumin and β2-microglobulin. Although the ISS remains prognostic within clinical trials, this system has limited utility for assessing risk on an individual patient basis and does not take into account genetic abnormalities and the role of intrinsic myeloma cell variability to allow for tailored therapy approaches. Importantly, the ISS and FISH/cytogenetic abnormalities can be combined in a prognostic model (Table 1).11 There are good data supporting the application of a combined approach for risk prognostication in the design of biomarker-focused clinical trials.30

GENE EXPRESSION PROFILING BIOMARKERS
Global gene expression profiling (GEP) is an alternative technology that integrates the influence of multiple genetic abnormalities on important cellular pathways associated with proliferation, differentiation, apoptosis, and other biologic features in a single signature. The University of Arkansas for Medical Sciences (UAMS) group was the first to define a 70-gene classifier (GEP70) characterizing 7 distinct gene expression clusters that identified patients with high risk for short progression-free survival (PFS) and overall survival (OS).31 The prognostic significance of the UAMS GEP70 assay has been validated in several studies performed independently by U.S. and international groups. In another study, GEPs obtained from patients with newly diagnosed MM included in the HOVON65/GMMG-HD4 trial were used to generate a prognostic signature of 92 genes (EMC-92-gene signature) capable of distinguishing between patients with high risk and low risk that was subsequently confirmed in independent validation sets of newly diagnosed and patients who relapsed.32 Other signatures linked to short survival have been defined, including a 17-gene signature identified by UAMS, a 15-gene signature in the IFM trials, and a 6-gene signature in the MRC Myeloma IX trial.33,34 Interestingly, the 17-, 15-, and 6-gene signatures do not share any common genes, reflecting variation in treatment strategies or patient selection between studies, or perhaps different aspects of myeloma biology. Although GEP provides valuable information regarding disease biology in the context of clinical trials, more work is required to define a standardized user-friendly GEP signature that can be more widely applied as a prognostic tool in clinics. To this end, the International Myeloma Working Group (IMWG) is conducting a study to unify the GEP signatures using prognostic modeling.

FLOW CYTOMETRIC BIOMARKERS AND MRD MONITORING
Flow cytometric methods have become an integral part of diagnosis and monitoring in many hematologic malignancies, such as acute and chronic leukemias; however, consensus regarding their routine use in plasma cell disorders is still evolving. Multiparameter flow cytometry (MFC) allows quantification and characterization of clonal plasma cells through their aberrant phenotypes rather than by light chain restriction. The immunophenotypic features of plasma cells vary depending on the diagnosis, stage of disease, and the type of therapies employed. Multiple studies have demonstrated the prognostic value of specific patterns of antigen expression by neoplastic plasma cells; for example CD19+, CD28+, CD81+, or CD117− expression has been associated with inferior outcome.35 Patient-specific immunophenotypic profiles with the sensitivity to detect one or more tumor cells in 10,000 normal cells make MFC an attractive strategy for MRD monitoring. From a clinical perspective, achieving an immunophenotypic CR predicts extended survival in younger patients undergoing intensive therapy and older patients treated with novel agents. The pitfalls of flow cytometric MRD assessment include its lack of standardization and the need for experienced personnel.36,37 A recent survey across 30 major medical institutions in the United States found that immunophenotypic features defining abnormal plasma cells varied substantially among institutions, as did the sensitivity of the MFC assays for MRD, which exhibited a 100-fold difference ranging from 0.0005% to 0.02%.38 The NCI, FDA, MMRF, and EuroFlow Consortium have initiated concerted efforts to overcome these drawbacks. In addition to MFC, polymerase chain reaction techniques are well standardized and highly sensitive (10−5 to 10−6), with several studies showing that, among patients achieving a CR, MRD-negative status is associated with substantial improvements in PFS and OS.39,40 However, designing allele-specific oligonucleotides for each individual is
laborious and time consuming, relatively expensive, and requires high-quality DNA, not only for post-treatment samples but also at baseline. Moreover, the genetic mutations are not stable longitudinally. Systematic assessment of MRD by next-generation sequencing (NGS) offers a novel platform with increased sensitivity, in particular for serial monitoring of mutational shifts between diagnosis and relapse.\textsuperscript{41} Progression is characterized by dynamic clonal equilibrium resulting from multiclonal presentation, clonal dominance, and post-treatment clonal selection. With respect to the potential need for frequent MRD sampling, the question of whether NGS of peripheral blood plasma cells could replace bone marrow testing is being studied. It should be noted that MRD negativity does not equate with complete tumor eradication, particularly in a disease that is typically characterized by patchy marrow infiltration and extramedullary involvement. In parallel with standardizing assays and validating prognostic MRD thresholds, there is an increasing interest in MRD monitoring as a tool for risk-adapted clinical trials.\textsuperscript{42}

\section*{IMAGING BIOMARKERS}
Paralleling the improvements in laboratory techniques, great advances have been made in imaging biomarkers. For a disease like MM that is characterized by bone involvement in at least 80\% of patients, imaging plays a critical role in diagnosis. The use of imaging biomarkers is as old as skeletal surveying itself; moreover, as the field of medical imaging has expanded to include MRI and PET, the number of available biomarkers has also increased. The principal imaging biomarkers are currently topographic markers such as lytic bone lesions and/or abnormal plasma cell proliferations in bone marrow or soft tissues. The major advantages of MRI and PET/CT compared with skeletal survey are discrimination between normal and invaded bone marrow, visualization of extramedullary disease (EMD) and cord involvement, and better sensitivity for lytic bone lesions. MRI and PET/CT biomarkers of focal marrow abnormalities (with or without osteolysis) provide a method to assess disease burden and prognosis and monitor response to therapy. The number of focal lesions at baseline on MRI or $^{18}$F-FDG PET/CT adversely affected both OS and event-free survival (EFS), as did the presence of EMD and failure of FDG suppression. Specifically, more than three baseline PET focal lesions and more than seven baseline MRI focal lesions (present in 32\% and 36\% of newly diagnosed patients with MM, respectively) were each associated with shorter EFS and OS.\textsuperscript{43-45} Moreover, complete suppression of FDG before the first ASCT conferred a favorable outcome.\textsuperscript{44} Additionally, the absence of PET suppression by day 7 of the first induction cycle in patients with MM who were treated in the Total Therapy 3 trials was associated with inferior OS and PFS.\textsuperscript{46} These observations have important implications and require further validation in the era of novel therapy induction regimens. PET/CT provides a complementary tool to biopsy for assessing heterogeneity and the effect of treatment within and across multiple disease sites. Emerging biomarkers, such as novel PET probes and dynamic contrast-enhanced (DCE) MRI parameters, including diffusion/perfusion, angiogenesis, and mismatches in tumor metabolism, provide a unique opportunity to evaluate response and resistance to antымyeloma therapy. Data demonstrating the efficacy of $^{11}$C-thymidine PET and $^{18}$F-fluorothymidine for response evaluation are promising.\textsuperscript{47} Although the newer imaging biomarkers have not yet been fully validated, recent advances in functional and molecular imaging provide a wealth of opportunities and accumulating data suggest that these biomarkers will become increasingly important in the future.

\section*{BONE TURNOVER BIOMARKERS}
Biomarkers of bone turnover (resorption and formation) are attractive noninvasive tools for detecting early bone involvement and for evaluating the risk of skeletal morbidity and response to antiresorptive treatment.\textsuperscript{48} These markers can be divided into two categories: collagen fragments released from the bone matrix during degradation, and enzymes released from either osteoblasts or osteoclasts. Biomarkers reflecting osteoclast-mediated degradation of collagen, including N-terminal cross-linking telopeptide of type-1 collagen (NTX), C-terminal cross-linking telopeptide of type-1 collagen (CTX), C-terminal cross-linking telopeptide of type-1 collagen generated by metalloproteinase (ICTP), and deoxyypyridinoline, provide information on the remodeling process and reflect whole-body bone turnover as opposed to local changes in skeletal homeostasis.\textsuperscript{49} Urinary NTX, serum CTX, and serum ICTP levels are elevated in patients with MM and correlate with advanced osteolytic disease.\textsuperscript{50-52} Furthermore, urinary NTX and serum ICTP correlate with risk for skeletal complications, PFS, and OS.\textsuperscript{51,53,54} Procollagen type-1 N-propeptide and procollagen type-1 C-propeptide signify new bone formation.\textsuperscript{55} Receptor activator of nuclear factor-kappa B ligand (RANKL) and osteoprotegerin are also important markers of bone turnover.\textsuperscript{54,56} Newer techniques using a label-free mass spectrometry-based strategy have been used to distinguish no versus high levels of bone disease.\textsuperscript{57} Results from ongoing trials evaluating biomarkers that mirror the dynamics of bone disease in MM will help to identify their true value in clinical practice.

\section*{EPIGENETIC BIOMARKERS}
There is now substantial evidence supporting the notion that epigenetic changes, including DNA methylation, histone acetylation, and microRNAs, are important for MM development and progression. Normal B cells, plasma cells, and MGUS cells have a methylation pattern that is distinct from that of malignant cells in patients with newly diagnosed MM and plasma cell leukemia.\textsuperscript{58} The transition from MM to plasma cell leukemia has been associated with promoter hypermethylation of genes involved in cell signaling and cell adhesion pathways, and with patterns of global hypomethylation.\textsuperscript{59,60} Unsupervised clustering of myeloma samples de-
BIOMARKERS FOR RISK OF PROGRESSION IN MYELOMA PRECURSORS

At present, there are no reliable biologic markers that predict which individuals with MGUS or SMM will progress to MM. In the absence of such markers, patients are risk stratified based on two commonly used models developed by the Mayo Clinic and the Spanish study group that are derived from clinical variables identified through retrospective studies. The Mayo Clinic model is based on quantification of M spike and bone marrow plasma cells, whereas the Spanish model includes the degree of clonality assessed by immunophenotyping (i.e., the balance between malignant and residual normal plasma cells). Recent studies indicate that chromosomal abnormalities are also critical determinants of the rate of progression in SMM. Two studies showed that the presence of del(17p) or t(4;14) is associated with the shortest time to progression and that trisomies and gains of 1q21 are risk factors for progression (Table 1). Genetic heterogeneity is established early during clonal plasma cell development. GEP has identified major differences between MGUS and normal plasma cells, yet no clear distinctions have emerged between MGUS and MM. The Arkansas group used GEP clustering in 351 patients with MM, 44 with MGUS, and 12 with SMM, and identified four major signatures; a MGUS-like signature in patients with MM was associated with improved survival. A recent prospective observational study showed that increased GEP70 score was an independent predictor of risk of progression from precursors to MM. Functional MRI incorporating DCE, diffusion weighting, or PET has the potential to detect small volumes of active tumor before morphologic changes become apparent. In a small study, DCE MRI microvascular parameters represented as rate constant (kep) and transfer constant (ktrans) showed moderate correlation with microvessel density (MVD) in patients with MGUS, SMM, and MM, whereas MVD increased progressively along the myeloma spectrum, recapitulating an angiogetic switch. The presence of MRI focal lesions is a predictor of early progression. In the largest study to date, involving 149 patients with SMM, focal marrow abnormalities were identified in 28% of subjects and the presence of two or more focal lesions was shown to have independent prognostic significance for progression to symptomatic disease.

A recently published trial of longitudinal MRI in untreated SMM patients revealed that MRI-progressive disease, as defined by new or increasing focal or diffuse bone marrow abnormalities, was associated with a 16.5-fold increased risk of progression to MM compared with MRI-stable disease (p < 0.0001). Although further technical refinement is needed, it is likely that biomarkers based on GEP, imaging, and immunophenotyping that can be followed in patients with MGUS or SMM will be introduced into the clinic, allowing us to better understand variations in sequential trajectories and predict those patients who are at high risk of disease progression and for whom treatment intervention is essential to prevent the emergence of serious end-organ damage.

CONSIDERATIONS ON DESIGNING BIOMARKER-FOCUSED CLINICAL TRIALS

Although it is currently feasible to define different risk groups using sets of prognostic biomarkers, we are not yet at the point where this can actually drive go/no-go decisions or support a specific alternative treatment strategy in a high-risk group or less intense therapy for those with favorable risk. Some might argue that this concept of optimization of therapy would not apply for an incurable malignancy, and that all patients should receive the optimal currently available treatment strategies (including ASCT) that have been tested in phase III clinical trials to achieve the best outcome. However, emerging evidence based on use of the best available treatments for newly diagnosed MM and SMM, together with refinements in risk categorization, clearly indicates that many patients have a greater than 50% chance of surviving more than 10 years and may not need up-front intensification of treatment. In contrast to standard-risk patients, for whom progress has been made in improving outcomes, clinical trials based on a ‘one-size-fits-all’ strategy have in general been disappointing for high-risk MM, which constitutes a distinct subgroup based on clinical and biologic biomarkers (Table 1). For this patient population the median survival has remained poor (approximately 2 to 3 years) despite aggressive therapy incorporating almost every available drug and treatment modality. In such cases, treatment intensification with dose-dense chemotherapy that is rotated and maintained for a long period of time to potentially induce consistent therapeutic pressure on the myeloma clone has not yielded encouraging results. An early IFM study compared the efficacy of autologous stem cell transplantation and ASCT in high-risk patients defined by high β2-microglobulin and del(13q) detected by FISH. Although the definition of high-risk disease has improved since the inception of this trial, no difference in EFS was seen between the two transplant types. The strategy of tandem ASCT followed by nonmyeloablative autologous transplant led to improved CR but did not translate into a survival benefit compared with double ASCT be-
cause of greater treatment-related mortality. Current studies are looking into immunomanipulation in the context of allogeneic transplantation. Tumor/stromal interactions, signaling pathway dependencies, and genetic and epigenetic alterations in MM provide novel targets for the development of drugs that are intended to abrogate malignant progression through specific drug protein interactions (Table 2; reviewed in Boyd et al.). Combinations of molecular targeted drugs or monoclonal antibodies and a tolerable conventional chemotherapy regimen seem promising, specifically for high-risk MM. As more effective treatment options become available, it is imperative to refine the yardstick for measuring the depth of responses that correlate with outcome. Once the definition of MRD in MM is standardized, the next generation of randomized studies should include MRD as a surrogate endpoint to assess which therapeutic strategy produces the deepest and most sustainable response.

The ability of high-throughput “omics” platforms to profile a large number of analytes in a single assay, together with the rapid expansion of next-generation sequencing for clinical use, is increasing the technical and logistical complexity of biomarker validation. As a result, biomarkers for identifying high-risk disease will undergo refinements and will need to be validated in order for us to screen an adequate number of patients for clinical trials focused on the subgroup of interest. Steps in the development, validation, and qualification of candidate biomarkers are shown in Fig. 1. The Clinical Laboratory Improvement Amendments (CLIA) approach suggests that the development of biomarkers should be performed in defined laboratories according to relevant standard operating procedures in order to establish consistency in sample handling, sample testing, assay interpretation, and reporting of results across laboratories. This implies that all biomarkers should be developed in CLIA-certified laboratories and in the context of clinical trials in which data are collected according to the principles of good clinical practice.

For a biomarker to be used to direct patient care, it must be shown to have clinical utility with very high levels of evi-

### TABLE 2. Biomarkers for Molecularly Targeted Therapies in Multiple Myeloma

<table>
<thead>
<tr>
<th>Alterations/Genes</th>
<th>Target/Biomarker</th>
<th>Prevalence</th>
<th>Prognosis</th>
<th>Targeted Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(4;14) FGFR3/MMSET</td>
<td>FGFR3 tyrosine kinase receptor</td>
<td>10-15%</td>
<td>Intermediate</td>
<td>PRO-001, CHIR 258, PKC412</td>
</tr>
<tr>
<td>t(14;16) c-MAF t(14;20) MAFB</td>
<td>MAF overexpression</td>
<td>5-10%</td>
<td>Poor</td>
<td>MEK inhibitors</td>
</tr>
<tr>
<td>t(11;14) CCND1 t(6;14) CCND3</td>
<td>Cyclins</td>
<td>19%</td>
<td>Standard</td>
<td>Cyclin D inhibitors</td>
</tr>
<tr>
<td>t(14;16) c-MAF</td>
<td>c-MYC</td>
<td>Poor</td>
<td>Bromodomain inhibitors e.g., JQ1</td>
<td></td>
</tr>
<tr>
<td>t(14;20) MAFB</td>
<td>MAF overexpression</td>
<td>5-10%</td>
<td>Poor</td>
<td>MEK inhibitors</td>
</tr>
<tr>
<td>8q24 translocations (c-MYC)</td>
<td>STAT3 and MEK/ERK signaling</td>
<td>39%</td>
<td>Poor</td>
<td>STAT3 and MEK inhibitors</td>
</tr>
<tr>
<td>+1q (CKS1B, PDZK1 and BCL9)</td>
<td>Early studies showed poor survival</td>
<td>Poor</td>
<td>Mutant R1 inhibitor</td>
<td></td>
</tr>
<tr>
<td>Deletion of 1p (FAF1 and CDKN2C)</td>
<td>-</td>
<td>11%</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Deletion of 13q (RB1)</td>
<td>-</td>
<td>45% by FISH and 19% by conventional cytogenetics</td>
<td>Poor</td>
<td></td>
</tr>
<tr>
<td>Deletion of 17p (TP53 and MDM2)</td>
<td>Mutant or WT TP53</td>
<td>10%</td>
<td>Poor</td>
<td>Nutlin, PRIMA-1, CHK inhibitors, and filanesib (target G2M)</td>
</tr>
<tr>
<td>Proliferative myeloma</td>
<td>Ki67, GEP-PR subtype</td>
<td>Poor</td>
<td>Spindle kinase inhibitors, Aurora kinase inhibitors</td>
<td></td>
</tr>
<tr>
<td>NF-κB pathway (multiple genes e.g., NFKB1, NFKB1, CYLD, TACI, NIK, TRAF2, TRAF3, BIRC2, BIRC3, VWOX, and CD40)</td>
<td>Gene expression signature</td>
<td>Poor</td>
<td>MLN220B (inhibitor of IKKβ)</td>
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</tr>
<tr>
<td>JAK/STAT pathway (CCND2)</td>
<td>Cyclins</td>
<td>50%</td>
<td>Poor</td>
<td>JAK inhibitors: atlimprimod, AZD480, T671209, and INC165662</td>
</tr>
<tr>
<td>MAPK/RAS pathway</td>
<td>RAS mutations (20-35%)</td>
<td>20-35% BRAF mutations (4%)</td>
<td>Poor</td>
<td>Farnesyl transferase inhibitors: perifosine, FTI-277, and tipifarnib. MEK inhibitors: AZD6244 and ASTO3026. BRAF kinase inhibitors</td>
</tr>
<tr>
<td>PI3 kinase pathway</td>
<td>Cyclins</td>
<td>PI3K inhibitors: SF1207, pimochemone, and CAL-101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epigenetic changes</td>
<td>Histone methyltransferase activity of MMSET</td>
<td>15%</td>
<td>HDAC6 inhibitor: ACY-1215</td>
<td></td>
</tr>
<tr>
<td>DNA methyltransferase inhibitors e.g., 5-azacytidine, 5-aza-2′-deoxycytidine</td>
<td></td>
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</tr>
</tbody>
</table>
Clinical utility refers to the ability of the biomarker to improve clinical decision-making and patient outcomes. Whereas a good predictive biomarker indicates who should receive the targeted treatment, a good prognostic biomarker does not necessarily have clinical utility. We already have several risk stratification biomarkers that are prognostic; however, these have not been validated for clinical utility. For example, even if the ISS distinguishes outcomes between three subgroups, clinical evidence supporting de-escalating or escalating treatment according to the risk group is not available. Improvement in patient outcomes can be achieved by conducting strategic clinical trials involving risk models based on multiple biomarkers.

Evaluation of prognostic biomarkers for clinical utility will require carefully designed clinical trials within the framework of carefully asked questions (Fig. 1). Prospective enriched integral biomarker trial designs that predefine an eligible population by the presence of a strong biomarker (i.e., HER2 in breast cancer or BCR-ABL in CML) may not be appropriate for testing a new treatment in patients with MM as positive results from such a study would leave one wondering whether the treatment might also have worked in the biomarker-negative subgroup. Moreover, selecting patients with, for example, BRAF-mutated tumors (which have a 4% incidence within MM) for anti-BRAF therapy presents a difficult challenge. Such a phase III trial would require screening of thousands of patients with MM to accomplish a BRAF-mutated phase III selection. Moreover, if we are to generate high-quality data supporting the magnitude of benefit of a biomarker for a patient, clinician, or third party payer, it is important to evaluate and report biomarker-focused clinical trials within the framework of defined guidelines such as REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) to ensure that all necessary information is included.82

The presence of clonal heterogeneity and subclonal evolution within a single patient across time (with treatment) and space (within medullary and extramedullary sites) has im-
important implications for the development of biomarkers. In the future, characterization of the size of the subclone carrying the target will very likely influence the choice of treatment because completely eradicating a clone that is present 90% of the time would be much more important than targeting a clone that is present only 5% of the time. In this regard, samples should be prospectively collected from large clinical trials, not only at the time of diagnosis but also longitudinally, for the creation of repositories to either validate existing prognostic markers/signatures or generate new ones. Several ongoing multinational efforts, such as the CoMMpass study, aim to provide a comprehensive understanding of MM in the era of novel agents.

CONCLUDING REMARKS
Studies performed over the last few years have emphasized that MM is a complex disease that is not amenable to treatment through a single therapeutic approach or inhibition of a single target or single pathway. A new generation of biomarkers based on cytogenetics, epigenetics, focal lesions, clone status, and GEP signature is beginning to provide clinicians with more information about the clinical behavior of the disease, providing a basis on which risk stratification models and therapies can be continuously refined. The new biomarker-based definition of MM has direct clinical implications for initiating treatment in a biomarker-positive subgroup. Further studies should apply risk stratification in the trial design to specifically answer questions regarding treatment within each risk group. Currently, few data are available to support the use of biomarker-guided treatment optimization, although with a robust pipeline of novel targeted agents and standardized definitions of MRD linked to traditional clinical endpoints, this remains an important goal of ongoing research. A systems-based approach including collaboration across multiple institutions and investigators will enhance our ability to conduct biomarker-focused clinical trials in a more effective manner.

Disclosures of Potential Conflicts of Interest

Relationships are considered self-held and compensated unless otherwise noted. Relationships marked “L” indicate leadership positions. Relationships marked “I” are those held by an immediate family member; those marked “B” are held by the author and an immediate family member. Institutional relationships are marked “Inst.” Relationships marked “U” are uncompensated.

Disclosure of Potential Conflicts of Interest


References


