Abstract

Remarkable progress has been achieved in multiple myeloma, and patient median survival has been extended 3- to 4-fold. Specifically, there have been 18 newly approved treatments for multiple myeloma in the past 12 years, including seven in 2015, and the treatment paradigm and patient outcome have been transformed. The definition of patients benefitting from these therapies has been broadened. Response criteria now include minimal residual disease (MRD), assessed in bone marrow by multicolor flow cytometry or sequencing, and by imaging for extramedullary disease. Initial therapy for transplant candidates is a triplet incorporating novel therapies—that is, lenalidomide, bortezomib, and dexamethasone or cyclophosphamide, bortezomib, and dexamethasone. Lenalidomide maintenance until progression can prolong progression-free and overall survival in standard-risk multiple myeloma, with incorporation of proteasome inhibitor for high-risk disease. Studies are evaluating the value of early versus late transplant and MRD as a therapeutic goal to inform therapy. In non-transplant patients, triplet therapies are also preferred, with doublet therapy reserved for frail patients, and maintenance as described above. The availability of second-generation proteasome inhibitors (carfilzomib and ixazomib), immunomodulatory drugs (pomalidomide), histone deacetylase inhibitors (panobinostat), and monoclonal antibodies (elotuzumab and daratumumab) allows for effective combination therapies of relapsed disease as well. Finally, novel therapies targeting protein degradation, restoring autologous memory anti–multiple myeloma immunity, and exploiting genetic vulnerabilities show promise to improve patient outcome even further. Clin Cancer Res; 22(22); 5419–27. ©2016 AACR. See all articles in this CCR Focus section, *Multiple Myeloma: Multiplying Therapies.*

Introduction

Over the past four decades, remarkable progress has been made in our understanding of the biology and pathogenesis of plasma cell dyscrasias. These advances have translated to evolving definitions of disease and prognosis; more stringent criteria for response; transformation of the treatment paradigm integrating stem cell transplantation, targeted therapies, and immune therapies; and most importantly, increased extent and frequency of response associated with 3- to 4-fold prolongation of median survival (Fig. 1; refs. 1–4). Indeed, subsets of patients with favorable genetic profiles now have a chronic disease with functional cure. This CCR Focus will highlight the landmarks of progress in disease biology and clinical practice and provide a roadmap for even further progress.

Definition of the Disease

As detailed by Landgren and Rajkumar (5), the definition of multiple myeloma has traditionally included excess monoclonal bone marrow plasma cells in the setting of monoclonal protein in blood and/or urine, and treatment was initiated only in the setting of disease-related manifestations, including hypercalcemia, renal dysfunction, anemia, or bone disease (CRAB). Individuals at earlier stages in the spectrum of plasma cell dyscrasias, namely monoclonal gamopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) with lower amounts of monoclonal protein and bone marrow plasma cells, were followed without therapy. At present, patients with MGUS are monitored expectantly, as overall, only 1% of individuals per year will develop multiple myeloma or a related lymphoproliferative disease; within MGUS, non-IgG monoclonal protein, monoclonal protein >1.5 g/dL, and abnormal k:λ ratio can further refine risk of progression (6). Importantly, a recent study has led to the redefinition and extension of which patients with multiple myeloma can benefit from therapy. In particular, a recent trial compared lenalidomide and dexamethasone versus no therapy in patients with SMM, and it showed prolongation of both progression-free survival (PFS) and overall survival (OS) in the treated cohort (7). For there to be an OS difference with short follow-up, some of these patients with SMM progressed very rapidly to active multiple myeloma. On the basis of the analysis of the subset of SMM with rapid progression, the International Myeloma Working Group (IMWG) has therefore now redefined patients with active multiple myeloma who can benefit from therapy to include those with >60% bone marrow plasma cells, k:λ ratio >100, and more than one bone lesion on a total body MRI or PET/CT scan, even in the absence of CRAB (8). Correspondingly, SMM now includes patients with >3 g/dL serum monoclonal protein, 10% to 60% monoclonal bone marrow plasma cells, and absence of bone disease on MRI or PET/CT scan. Although the standard of practice is no therapy for patients with SMM, targeted and immune therapies used to treat active disease are well tolerated with a favorable therapeutic index. Therefore, multiple protocols of targeted and immune therapies are now ongoing treating patients earlier in the disease course to delay or prevent progression of SMM to
active disease requiring therapy. For example, a peptide-based vaccine targeting SLAMF-7, CD138, and XBP-1 in patients with SMM can generate an autologous immune anti–multiple myeloma response, which can be enhanced and shifted to memory response by lenalidomide or histone deacetylase (HDAC) 6 inhibitor (9–11). This early experience suggests that it might be possible to vaccinate patients, even at the MGUS phase, and generate an autologous memory anti–multiple myeloma response, which will prevent progression to disease requiring therapy.

Definitions of Response

As novel single-agent and combination therapies have improved and achieved increased extent and frequency of response in multiple myeloma, the definitions of response have similarly evolved. For example, complete response (CR) previously required the absence of monoclonal protein by immunofixation; a stringent CR in addition required normal κ/λ ratio. More recently, the IMWG has incorporated multicolor flow immunofluorescence flow cytometry and gene sequencing with sensitivity of up to 10^-6 into multiple myeloma response criteria, as well as absence of bone disease on more sensitive imaging, including MRI and PET/CT scanning (12). Recent meta-analyses and randomized trials are defining absence of minimal residual disease (MRD) using these metrics (13, 14). For example, early analyses from our trial of lenalidomide, bortezomib, and dexamethasone followed by early versus late high-dose therapy and autologous stem cell transplant and 1 year of lenalidomide maintenance therapy show that gene sequencing may be more sensitive than multicolor flow cytometry for detecting MRD and predicting outcome (15). Additional ongoing studies and more follow-up are necessary to achieve the important goal of defining the regulatory and clinical utility of MRD. Indeed, initial combination targeted therapy with or without transplant now achieves PFS of many years, highlighting the urgent need to establish the utility of MRD at earlier time points to predict PFS and OS so that it can
be used an endpoint in clinical trials for regulatory approval. Whether MRD negativity should be a goal of therapy for newly diagnosed or relapsed multiple myeloma and whether duration of maintenance therapy can be informed by MRD status are among the important patient management issues now being addressed in clinical trials. Already, patient-and study-level meta-analyses of available studies show that MRD negativity portends longer PFS and OS (14) and will likely be incorporated soon in regulatory approval processes of novel agents and medical practice in multiple myeloma. Finally, rapid progress in novel technologies for measuring MRD includes single multiple myeloma cell gene sequencing and serum cell-free DNA, “or liquid biopsies,” which may make it possible to more accurately and readily measure MRD (16, 17).

Genetic and Molecular Pathogenesis: Prognostic and Therapeutic Implications

Our understating of the genetic and molecular pathogenesis of multiple myeloma has similarly rapidly advanced. Early genomic profiling studies characterized changes associated with progression from MGUS to SMM to active multiple myeloma, showing that many hallmark abnormalities are present even at the MGUS phase (18). More recent genomic studies involving large numbers of clinically annotated patient samples have delineated heterogeneity and clonality at the time of diagnosis and relapse of multiple myeloma, defined mechanisms of sensitivity or resistance to targeted therapies, identified novel targets, and allowed for individualized treatments (19–22). In this CCR Focus, Szalat and colleagues (23) will detail these complexities and describe the utility of genomic profiling in clinical practice today, as well as prospects for precision medicine in multiple myeloma.

Prognostication in multiple myeloma has evolved in parallel with the development of more effective therapies. Traditionally, the International Staging System (ISS) of the IMWG has staged patients based upon serum β2 microglobulin and albumin, as these parameters are readily and universally obtainable. Correlation of FISH analyses with clinical outcome has identified standard-risk multiple myeloma with t(1;14) translocation and hyperdiploidy versus high risk multiple myeloma with t(4;14), t(14;16), t(14;20), del(17p), and del(13q14). Most recently, the IMWG has incorporated FISH into the ISS (24). Importantly, the definitions of standard- versus high-risk disease are continuing to evolve with improved therapies. For example, bortezomib can overcome the adverse prognosis conferred by t(4;14) in patients treated with conventional chemotherapy (25). Pomalidomide and the monoclonal antibodies (mAb) elotuzumab and daratumumab can achieve responses even in the context of del(17p) (26). As multiple myeloma progresses, the majority of patients acquire high-risk genetic features from evolution and/or expansion of most resistant clones. Patterns of clonal changes observed at the time of relapse compared with diagnosis include no change, linear evolution, differential clonal response, or branching evolution (21). These changes are related to intrinsic genomic changes, as well as the influence of the tumor microenvironment and treatment. Most importantly, combinations of targeted agents with different mechanisms of action—including proteasome inhibitors (PI) with immunomodulatory drugs (IMiD; refs. 27–29), PIs with HDAC inhibitors (30, 31), and IMiDs with mAbs (32–34)—can overcome these adverse features and achieve durable responses. For example, patients with high-risk 17p(p53) deletion multiple myeloma can respond to the second-generation IMiD pomalidomide, the second-generation PI carfilzomib, or the combination, and daratumumab achieves responses as a single agent and in combination with IMiDs or PIs in multiply relapsed far advanced disease. As combination-targeted and immune therapies now come into routine clinical practice in multiple myeloma, we will need, in on ongoing fashion, to define which patient subgroups benefit from particular therapies, and conversely, which patients remain high risk and in need of new options. As multiple myeloma is an orphan disease and is itself very heterogeneous, international registries of genomically profiled and clinically annotated patient databases will be necessary to provide the most effective combination therapies to individual patients at distinct time points in their disease course.

Although we and others are attempting to selectively target mutations (BRAF; ref. 35) and pathways (MEK or ERK; ref. 36) in multiple myeloma, the heterogeneity and complexity of genomic abnormalities and multiclonality right from diagnosis, coupled with ongoing DNA damage, are major obstacles to the goal of precision medicine in multiple myeloma. As noted above, multiple myeloma is a heterogeneous orphan disease, and pooling of large amounts of genomically profiled and clinically annotated data will be necessary to better define patient subsets and inform therapy targeting genetic abnormalities/pathways intrinsic to the tumor cell; such efforts are now ongoing. We are also undertaking an alternative strategy to target the biologic sequelae of the constitutive and ongoing genetic instability and DNA damage in multiple myeloma. For example, multiple myeloma cells with decreased YAP1 copy number do not die despite constitutive and ongoing DNA damage: in this subset, genetic deletion of STK4 restores YAP1 expression and P73-mediated apoptosis, even in the setting of p53 deleted multiple myeloma (Fig. 2; ref. 37). These studies provide the rationale for the first kinase inhibitor trial targeting STK4 in this subset of patients with multiple myeloma. We have also shown that multiple myeloma cells with amplification of Myc have very high levels of replicative stress and reactive oxygen species (ROS; ref. 38); our preclinical studies show that combining agents that block stress response (i.e., ATR inhibitor) with those enhancing ROS (i.e., bortezomib) achieves synergetic cytotoxicity, setting the stage for combination clinical trials in this subset of multiple myeloma patients with poor prognosis. Finally, we and others are now attempting to target not only individual genetic abnormalities, but also aberrant regulatory loops in multiple myeloma. For example, we have recently shown that increased KDM3A demethylase activity in multiple myeloma allows for transcriptional activation of the IRF4–KLF2 axis in multiple myeloma, of central importance due to the its hallmark role in promoting homing of multiple myeloma cells to the bone marrow and multiple myeloma cell survival. Conversely, targeting increased KDM3A demethylase activity restores methylation of the IRF4 and KLF2 promoters and suppresses related gene transcription, thereby inhibiting multiple myeloma cell survival (39). These examples suggest the promising therapeutic potential of targeting biological consequences of genomic/epigenomic abnormalities in multiple myeloma.

Evolution of Therapy in Multiple Myeloma

The modern history of therapy in multiple myeloma begins with melphalan and prednisone in the 1960s and evolved to combination therapy in the 1970s. High-dose therapy
followed by bone marrow and then peripheral blood stem cell rescue were major advances in the 1980s and 1990s, which extended median PFS to 4 to 5 years. Since the late 1990s, there has been a revolution in multiple myeloma therapy with the development of the PIs bortezomib (40), carfilzomib (41), ixazomib (42), and marizomib (43); the IMiDs lenalidomide and pomalidomide (44–47); the mAbs elotuzumab and daratumumab (32–34); and the HDAC inhibitor panobinostat (30). These advances are the direct result of collaborative, bench-to-bedside translational studies involving academia, the pharmaceutical industry, regulatory authorities, NIH funding sources, and patient advocacy organizations. Over the past four decades, we and others have developed laboratory and animal models of multiple myeloma in the bone marrow, which have identified molecular and biological mechanisms mediating tumor growth, survival, and drug resistance and also been useful to validate novel targeted therapies (48–53). These fundamental studies both enhanced our understanding of multiple myeloma pathogenesis and provided novel targets for drug discovery and development, including cell-surface antigens and receptors (54–56), signaling cascades (57–61), cytokines (62), and bone marrow accessory cells (63–66). Importantly, this pioneering work validated targeting the symbiotic heterotypic interactions between the tumor cell and its microenvironment to overcome drug resistance and improve patient outcome. PIs and IMiDs are the prototype drug classes targeting the multiple myeloma cell, tumor cell interaction with the bone marrow, and the bone marrow microenvironment, and multiple myeloma now represents a model for the therapeutic importance of targeting the tumor cell in its microenvironment.

To date, these studies have translated to clinical trials resulting in 18 new FDA-approved regimens, which have transformed the treatment paradigm. Although novel agents are often initially evaluated in patients with relapsed and refractory multiple myeloma, their regulatory approval and clinical use now extends to newly diagnosed and relapsed multiple myeloma, as well as for maintenance therapy. Moreover, scientifically informed combination therapies (lenalidomide, bortezomib, and dexamethasone) have achieved unprecedented extent and frequency of durable responses and have established a new standard of care. These remarkable advances, and integration of these novel therapies into clinical practice, will be detailed by Orlowski and Lonial (67) in this CCR Focus.

**Therapies targeting protein homeostasis**

A hallmark advance in multiple myeloma was the preclinical and clinical development of the PI bortezomib (68–73). Its use was initially predicated upon blocking the degradation of IκB, preventing its dissociation from the NFκB subunits, and thereby blocking NFκB activation in multiple myeloma. Inhibiting NFκB was thought to be key, given that it mediates survival and drug resistance in multiple myeloma, modulates adhesion molecules on the tumor cell and in the microenvironment, and regulates transcription of multiple myeloma growth and survival cytokines in the bone marrow milieu. Subsequently, it has been shown to
In the ubiquitin–proteasome system, multiple proteasome inhibitors, including bortezomib, carfilzomib, ixazomib, and oproazib, primarily inhibit the chymotryptic-like proteasome activity, whereas marizomib inhibits chymotryptic-, tryptic-, and caspase-like activities. In the ubiquitin–proteasome cascade of protein degradation, ubiquitin proteasome receptors and deubiquitylating enzymes are upstream of the proteasome and required for recruiting and deubiquitylating ubiquitylated misfolded proteins, respectively, so that they can bind to the 20S core of the proteasome and be degraded. Blockade of either ubiquitin proteasome receptors or deubiquitylating enzymes upstream of the proteasome therefore has the potential to overcome proteasome inhibitor resistance. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ub, ubiquitin.

Figure 3. Targeting the ubiquitin proteasome system. Multiple proteasome inhibitors, including bortezomib, carfilzomib, ixazomib, and oproazib, primarily inhibit the chymotryptic-like proteasome activity, whereas marizomib inhibits chymotryptic-, tryptic-, and caspase-like activities. In the ubiquitin–proteasome cascade of protein degradation, ubiquitin proteasome receptors and deubiquitylating enzymes are upstream of the proteasome and required for recruiting and deubiquitylating ubiquitylated misfolded proteins, respectively, so that they can bind to the 20S core of the proteasome and be degraded. Blockade of either ubiquitin proteasome receptors or deubiquitylating enzymes upstream of the proteasome therefore has the potential to overcome proteasome inhibitor resistance. ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ub, ubiquitin.

Immune therapies in multiple myeloma

Immune therapies to overcome drug resistance in multiple myeloma will be described by Kumar and Anderson (86) in this CCR Focus. Immune therapies in multiple myeloma currently
include IMiDs, mAb-based therapies (Fig. 4), checkpoint inhibitors, HDAC inhibitors, vaccines, and cellular therapies. IMiDs directly trigger multiple myeloma cell apoptosis, abrogate tumor cell adhesion to the bone marrow, modulate cytokines, and inhibit angiogenesis as well as augment T-cell, natural killer (NK) cell, and NKT cell function while downregulating regulatory T cells (87–89). Binding to CRBN has been implicated in both direct cytotoxic- and immune-related effects of IMiDs. Importantly, they augment antibody-dependent cellular cytotoxicity and clinical activity of mAbs, and elotuzumab, which targets SLAMF-7, is approved in combination with lenalidomide to treat relapsed/refractory multiple myeloma (32, 54). Multiple myeloma cells—as well as myeloid-derived suppressor cells (MDSC) and plasmacytoid dendritic cells (pDC), which augment multiple myeloma cell growth and suppress host immune function—express PD-L1 (63–65), whereas immune effector T and NK cells express PD-1. Checkpoint blockade can induce multiple myeloma cell-specific CD4 and CD8 cytolytic T cells as well as NK-cell cytotoxicity, even in the presence of MDSCs and pDCs, thereby inhibiting multiple myeloma cell growth in the bone marrow milieu (65). Early studies show clinical efficacy of lenalidomide with PD-1 blockade (90). In preclinical studies, HDAC6 inhibitor can augment autologous multiple myeloma cytotoxicity and add to mAb and PD-L1 antibody. Peptide-based vaccine strategies are being evaluated to target multiple tumor-associated antigens on multiple myeloma cells and block progression of SMM to active disease (9–11), and multiple myeloma cell/DC fusion vaccines are being evaluated to treat MRD posttransplant and improve outcome (91). In both cases, multiple myeloma-specific T-cell immune responses have been triggered by vaccination, which can be increased by lenalidomide (90): a randomized trial comparing lenalidomide versus lenalidomide plus multiple myeloma cell/DC fusion vaccine posttransplant is ongoing. Excitingly, combination of vaccination with checkpoint inhibitors and HDAC6 inhibitor can promote effector T- and memory T-cell function and increased anti–multiple myeloma immunity, evidenced by cytotoxic activities, trigger production of Th1-type of cytokines (IFNγ, IL2, TNFα), and induce costimulatory/activation molecules. Their potential role for epitope spreading to allow for targeting additional tumor-associated antigens and enhance antitumor cytotoxic activities is under investigation. Finally, chimeric antigen receptor (CAR) T cells and other strategies, including immunotoxins, bispecific mAbs, and CAR T cells targeting BCMA, have demonstrated preclinical activity and early clinical promise (56, 92). In the future, it is likely that combinations of immune approaches, including IMiDs, mAbs, checkpoint inhibitors, HDAC inhibitors, vaccines, and/or cellular therapies, will confer long-lasting anti–multiple myeloma immunity and durable response.

Conclusions

Remarkable progress in multiple myeloma has been achieved in the past two decades, particularly due to integration of stem cell transplantation with novel therapies, including IMiDs and PIs. Most recently, the advent of mAbs has provided effective therapy even in multiply relapsed disease. Importantly, combination IMiD, PI, and mAb regimens are now being evaluated earlier in the disease course and will have even greater efficacy as initial therapy. The future is even more exciting. The three Achilles’ heels, or vulnerabilities, to exploit in novel therapeutics include blocking protein degradation, restoring anti–multiple myeloma immunity, and targeting the consequences of the constitutive genetic complexity and ongoing DNA damage, as described above. On the one hand, scientific advances will continue to increase our basic understanding and therapeutic armamentarium, allowing for clinical trials of precision medicine in multiple myeloma. Although these trials are promising, it may be difficult to target multiple, continually evolving clonal and genetic abnormalities...
in the right combination, at the right time, and in the correct sequence. That is why targeting the consequences of this ongoing DNA damage, such as blocking stress responses in multiple myeloma cells, offers great appeal. On the other hand, immune therapies are selective, adaptable, and potent and offer great promise to overcome ongoing genomic instability underlying relapse in multiple myeloma. As described above, the immune-based therapies in multiple myeloma now include IMiDs, mAbs, checkpoint inhibitors, HDAC6 inhibitors, vaccines, and cellular therapies. Importantly, preclinical and early clinical trials suggest the potency of combinations, such as IMiDs with mAbs, IMiDs with checkpoint inhibitors, IMiDs with HDAC6 inhibitors, and vaccines with IMiDs. Particularly exciting is early evidence that vaccinating patients with SMMA can induce an autologous anti–multiple myeloma selective response, which can be amplified and be of central and effector memory type in the presence of IMiDs and HDAC6 inhibitors. Ultimately, combinations of immune therapies used early in the disease course, now in SMMA and in the future in MGLIS, may achieve long-term memory anti–multiple myeloma immunity, which will prevent progression to active multiple myeloma ever requiring therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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