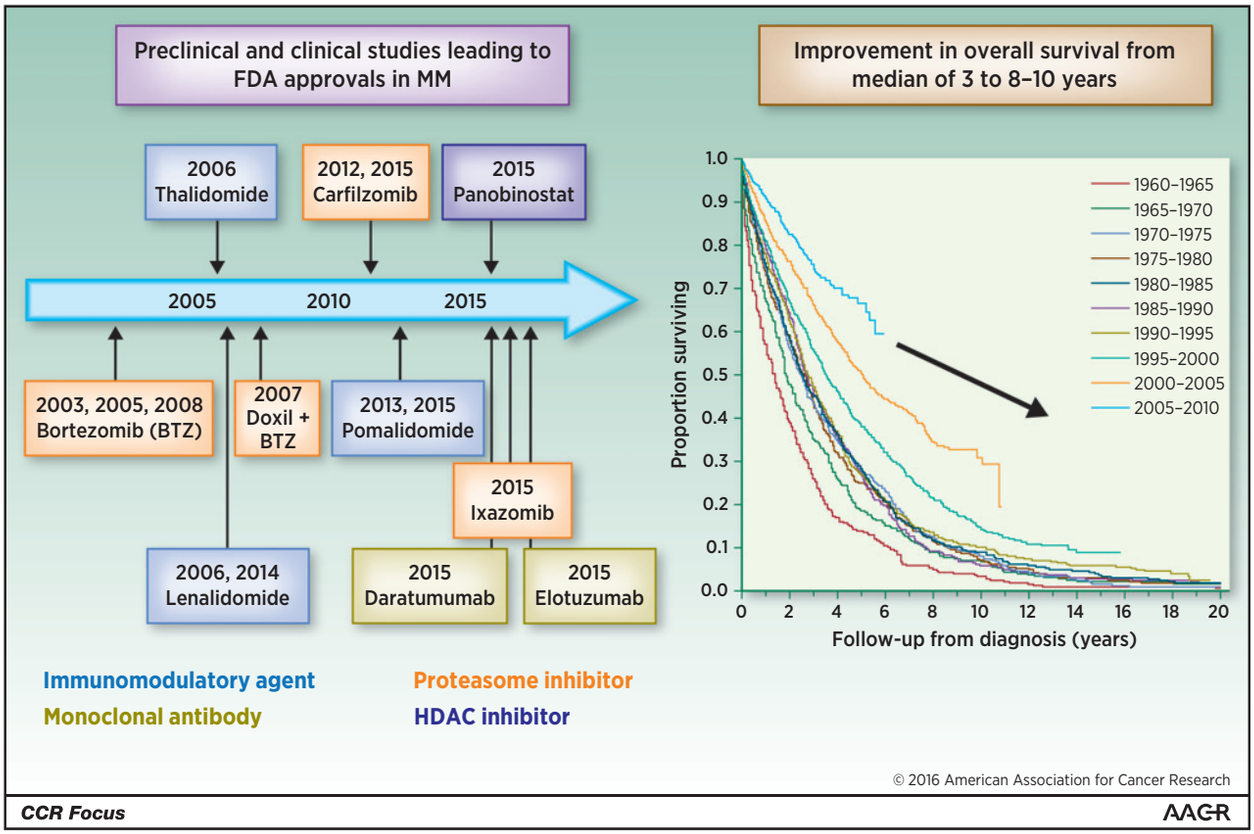

Figure 1.

Bench-to-bedside translation of novel agents in myeloma. Early advances in myeloma therapy included melphalan and prednisone, followed by combination chemotherapy and then high-dose melphalan, rescued first by bone marrow and more recently by peripheral blood stem cell transplantation. Importantly, remarkable progress has been made in the past 12 years due to the FDA approval of the proteasome inhibitors bortezomib, carfilzomib, and ixazomib; the immunomodulatory drugs thalidomide, lenalidomide, and pomalidomide; the histone deacetylase (HDAC) inhibitor panobinostat; as well as the monoclonal antibodies elotuzumab and daratumumab (left). All of these recent therapies have been initially evaluated and achieved responses in relapsed refractory multiple myeloma (MM), and then moved into clinical trials earlier in the disease course where their efficacy improved. Moreover, their use in combination—that is, lenalidomide, bortezomib, and dexamethasone—can achieve unprecedented frequency and extent of response when used as initial therapy. They have been integrated into the treatment paradigm of transplant candidates and nontransplant candidates as initial and maintenance therapies. As a consequence of these advances, overall survival (OS) has been extended from a median of 3 to 8–10 years (4), and the benefit of the most recently approved drugs will further improve outcome (right).

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(11;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 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 mechansims 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 genetic 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 synergistic 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

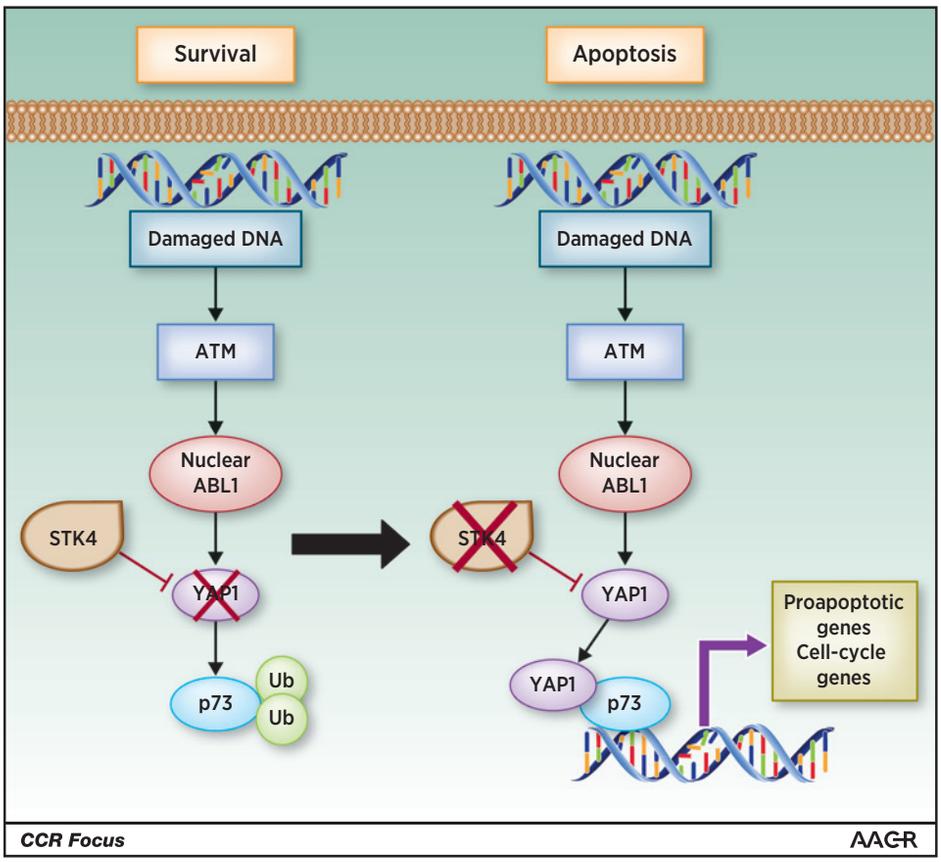

Figure 2.
Restoring apoptotic signaling by serine threonine kinase (STK) inhibition in YAP1-deficient multiple myeloma. A subset of patients with myeloma, lymphoma, and leukemia has decreased copy number and expression of YAP1 (37). As a result, these tumor cells with ongoing DNA damage do not undergo apoptosis (left). STK4 inhibits expression of YAP1; conversely, genetic or pharmacologic inhibition of STK4 allows reexpression of YAP1 and downstream p73-mediated apoptosis of these tumor cells with ongoing DNA damage to occur (right). Adapted from Cottini et al. (37).

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 have 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

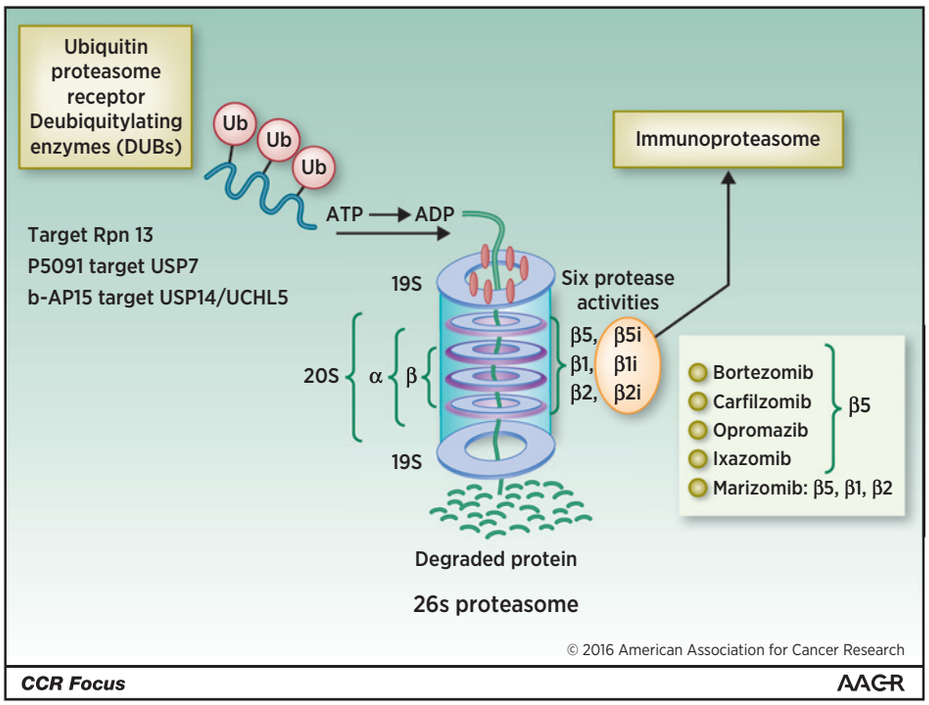

Figure 3.
Targeting the ubiquitin proteasome system. Multiple proteasome inhibitors, including bortezomib, carfilzomib, ixazomib, and opromazib, primarily inhibit the chymotryptic-like proteasome activity, whereas marizomib inhibits chymotryptic-, tryptic-, and caspase-like activities. In the ubiquitin–proteasome 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.

have multiple effects on multiple myeloma cells, including inducing ROS, triggering unfolded protein and stress responses, targeting the cell cycle, and triggering apoptosis, as well as in the microenvironment, including inducing osteoclast apoptosis and inhibiting angiogenesis. Through collaborative studies, bortezomib was rapidly translated to approval and use in relapsed and refractory multiple myeloma, in newly diagnosed disease, and as maintenance therapy, thereby establishing proof of principle for therapeutic targeting of protein degradation in multiple myeloma and other cancers.

Although bortezomib is now incorporated into multiple initial, relapse, and maintenance regimens in multiple myeloma, development of resistance to bortezomib is common, and ours and others' efforts are now focusing on delineating and targeting alternative mechanisms that modulate protein homeostasis. Second-generation PIs [carfilzomib (41), ixazomib (42), and marizomib (43)] have been preclinically and clinically validated (Fig. 3). Carfilzomib is an epoxyketone, irreversible, covalent PI without significant neurologic toxicity, which is approved alone and in combination with lenalidomide to treat relapsed multiple myeloma (39). Ixazomib is an oral, next-generation, boronic acid–based PI, which targets chymotryptic activity and can overcome bortezomib resistance; combined with lenalidomide and dexamethasone, it is approved to treat relapsed multiple myeloma and an effective all oral PI and IMiD regimen for newly diagnosed multiple myeloma (42, 74–76). Marizomib is a broad PI that targets chymotryptic, tryptic, and caspase activities, which in preclinical studies can overcome bortezomib resistance and has now translated to combination clinical trials with pomalidomide (43, 77).

We are attempting to overcome PI resistance using two strategies. First, we have shown in preclinical studies that targeting the (UPS) upstream of the proteasome at the level of the ubiquitin proteasome receptor or the deubiquitylating enzymes (DUB) can overcome PI resistance (Fig. 3). For example, we have defined the functional role of the DUBs USP7 (78) and USP14/UCHL5 (79) and the ubiquitin receptor Rpn13 (80) in multiple myeloma, shown that targeted inhibitors can overcome PI resistance, and translated these studies to a clinical trial of b-AP15/VLX 1570 targeting USP14/UCHL5 in multiple myeloma. Second, we have delineated mechanisms of the alternative aggresomal mechanism of protein degradation and shown that it is upregulated upon proteasome inhibition. Our preclinical studies combining the nonselective HDAC inhibitors vorinostat and panobinostat to block aggresomal degradation, together with bortezomib to block proteasomal degradation, triggered accumulation of ubiquitinated proteins and overcame PI resistance (81), setting the stage for derived clinical trials and the approval of panobinostat in combination with bortezomib to treat relapsed/refractory multiple myeloma (30). As HDAC6 binds to ubiquitinated protein on the one hand and to dynein motility complexes on the other, shuttling ubiquitinated protein to the aggresome for degradation, we developed and translated selective HDAC6 inhibitors to promising combination clinical trials in relapsed/refractory multiple myeloma, with improved tolerability relative to more broad HDAC inhibitors (82).

Finally, IMIDs target cereblon (CRBN) ubiquitin 3 ligase complex, resulting in the degradation of IZF1/3 and hallmark IRF4 and c-Myc in multiple myeloma (83, 84). Based upon this principle, we are now synthesizing degronimids, agents that both bind and activate ubiquitin 3 ligases and link to substrates, thereby allowing for proteasomal degradation of selective hallmark pathogenic proteins in cancer and other diseases (85).

Immune therapies in multiple myeloma

Immune strategies 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

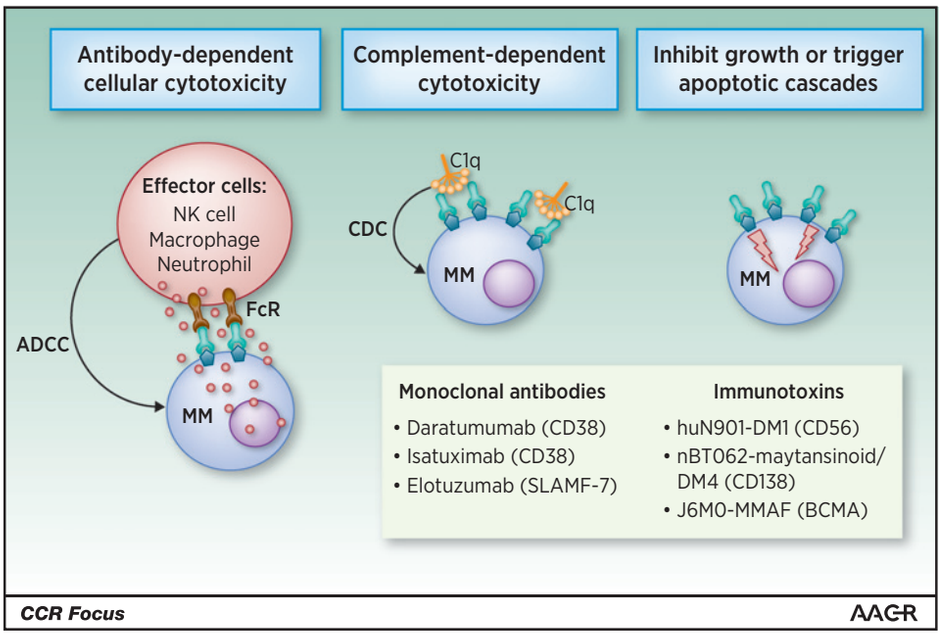

Figure 4.
mAb-based therapies for multiple myeloma (MM). mAbs used therapeutically in MM can trigger antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC); block signaling pathways mediating MM cell growth and survival and drug resistance; or trigger apoptotic signaling cascades. The FDA-approved mAbs daratumumab and elotuzumab target CD38 and SLAMF-7, respectively. NK, natural killer. Adapted from Tai and Anderson (93).

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, bispecifc 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 SMM can induce an autologous anti–multiple myeloma selective response, which can be augmented 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 SMM and in the future in MGUS, 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.

Grant Support

This work is supported by NIH/NCI grants RO-1 CA207237, RO-1 CA050947, RO-1 CA178264, P50 CA100707, PO-1 CA155258, and P30 CA006516 (to K.C. Anderson). K.C. Anderson is an American Cancer Society Clinical Research Professor.

Received August 17, 2016; revised September 20, 2016; accepted September 22, 2016; published online November 15, 2016.

References

1. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med 2011;364:1046–60.
2. Anderson KC. The 39th David A. Karnofsky lecture: bench-to-bedside translation of targeted therapies in multiple myeloma. J Clin Oncol 2012;30:445–52.
3. Anderson KC, Alsina M, Atanackovic D, Biermann JS, Chandler JC, Costello C, et al. NCCN guidelines insights: multiple myeloma, Version 3.2016. J Natl Compr Canc Network 2016;14:389–400.
4. Kumar SK, Dispenzieri A, Lacy MQ, Gertz MA, Buadi FK, Pandey S, et al. Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients. Leukemia 2014;28:1122–8.
5. Landgren O, Rajkumar SV. New developments in diagnosis, prognosis, and assessment of response in multiple myeloma. Clin Cancer Res 2016;22:5428–33.
6. Rajkumar SV, Kyle RA, Therneau TM, Melton LJIII, Bradwell AR, Clark RJ, et al. Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. Blood 2005;106:812–7.
7. Mateos MV, Hernández MT, Giraldo P, de la Rubia J, de Arriba F, López Corral L, et al. Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. N Engl J Med 2013;369:438–47.
8. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International myeloma working group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014;12:e538–e548.
9. Bae J, Smith R, Daley J, Mimura N, Tai Y-T, Anderson KC, Munshi NC. Myeloma-specific multiple peptides able to generate cytotoxic T lymphocytes: a potential therapeutic application in multiple myeloma and other plasma cell disorders. Clin Can Res 2012;17:4850–60.
10. Bae J, Rao P, Voskertchian A, Brown A, Maguire C, Richardson P, et al. A multiepitope of XBP-1, CD138, and CS1 peptides induces myeloma-specific cytotoxic T lymphocytes in T cells of smoldering myeloma patients. Leukemia 2015;29:218–29.
11. Bae J, Keskin D, Cowens K, Lee A-H, Dranoff G, Munshi NC, et al. Lenalidomide polarizes Th1 specific anti-tumor response and expands XBP1 antigen-specific central memory CD3+CD8+ T cells against various solid tumors. Leukemia. In press.
12. Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol 2016;17:e328–46.
13. Paiva B, van Dongen JJ, Orfao A. New criteria for response assessment: role of minimal residual disease in multiple myeloma. Blood 2015;125:3059–68.
14. Munshi NC, Avet-Loiseau H, Rawstron AC, Owen RG, Child JA, Thakurta A, et al. Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: a meta-analysis. JAMA Oncol 2016 Sep 15. [Epub ahead of print].
15. Avet-Loiseau H, Corre J, Lauwers-Cances V, Chretien M-L, Robillard N, Leleu X, et al. Evaluation of minimal residual disease (MRD) by next generation sequencing (NGS) Is highly predictive of progression free survival in the IFM/DFCI 2009 trial. Blood 2015;126;191.
16. Vij R, Mazumder A, Klinger M, O'Dea D, Paasch J, Martin T, et al. Deep sequencing reveals myeloma cells in peripheral blood in majority of multiple myeloma patients. Clin Lymphoma Myeloma Leuk 2014;14:131–9.
17. Hocking J, Mithraprabhu S, Kalff A, Spencer A. Liquid biopsies for liquid tumors: emerging potential of circulating free nucleic acid evaluation for the management of hematologic malignancies. Cancer Biol Med 2016;13:215–25.
18. Davies FE, Dring AM, Li C, Rawstron AC, Shammas MA, Fenton JAL, et al. Insights into the multipstep transformation of MGUS to myeloma using microarray expression analysis. Blood 2003;102:4504–11.
19. Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, et al. Initial genome sequencing and analysis of multiple myeloma. Nature 2011;471:467–72.
20. Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D, et al. Multiple myeloma research consortium, Getz G, Golub TR. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. Cancer Cell 2014;25:1–10.
21. Bolli N, Avet-Loiseau H, Wedge DC, van Loo P, Alexandrov LB, Martincorena I, et al. Heterogeneity of somatic mutations, clonal architecture and genomic evolution in multiple myeloma. Nat Commun 2014;5:2997.
22. Rashid N, Sperling A, Bolli N, Wedge D, Van Loo P, Tai Y-T, et al. Differential and limited expression of mutant alleles in multiple myeloma. Blood 2014;124:3110–7.
23. Szalat R, Avet-Loiseau H, Munshi NC. Gene expression profiles in myeloma: ready for the real world?. Clin Cancer Res 2016;22:5434–42.
24. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised International Staging System for Multiple Myeloma: a report from IMWG. J Clin Oncol 2015;26:2863–9.
25. Sonneveld P, Avet-Loiseau H, Lonial S, Usmani S, Siegel D, Anderson KC, et al. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the international myeloma working group. Blood 2016;127:2955–62.
26. Richardson P, Siegel D, Vij R, Hofmeister CC, Baz R, Jagannath S, et al. Pomalidomide alone or in combination with low-dose dexamethasone in relapsed and refractory multiple myeloma: a randomized phase 2 study. Blood 2014;123:1826–32.
27. Richardson PG, Weller E, Lonial S, Jakubowiak AJ, Jagannath S, Raje NS, et al. Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood 2010;116:679–86.

28. Richardson P, Weller E, Jagannath S, Avigan DE, Alsina M, Schlossman RL, et al. Multicenter, phase 1, dose escalation trial of lenalidomide plus bortezomib for relapsed and relapsed, refractory multiple myeloma. J Clin Oncol 2009;27:5713–19.
29. Richardson PG, Xie W, Jagannath S, Jakubowiak A, Lonial S, Raje NS, et al. Phase II trial of lenalidomide, bortezomib, and dexamethasone in patients with relapsed and relapsed/refractory multiple myeloma. Blood 2014;123: 1461–9.
30. San-Miguel JF, Hungria VT, Yoon SS, Beksac M, Dimopoulos MA, Elghandour A, et al. Panobinostat plus bortezomib and dexamethasone versus placebo plus bortezomib and dexamethasone in patients with relapsed or relapsed and refractory multiple myeloma: a multicentre, randomised, double-blind phase 3 trial. Lancet Oncol 2014;15:1195–206.
31. Dimopoulos M, Jagannath S, Yoon S-Y, Siegel DX, Lonial S, Qi J, et al. Vantage 088: an international, multicenter, randomized double-blind study of vorinostat (MK-0683) or placebo in combination with bortezomib in patients with multiple myeloma. Clin Lymphoma Myeloma Leuk 2016;16:329–34.
32. Lonial S, Dimopoulos M, Palumbo A, White D, Grosicki S, Spicka I, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. N Engl J Med 2015;373:621–31.
33. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. N Engl J Med 2015;373:1207–19.
34. Lonial S, Weiss BM, Usmani SZ, Singhal S, Chari A, Bahlis NJ, et al. Daratumumab monotherapy in patients with treatment-refractory multiple myeloma (SIRIUS): an open-label randomised, phase 2 trial. Lancet 2016;387:1551–60.
35. Andrulis M, Lehners N, Capper D, Penzel R, Heining C, Huellein J, et al. Targeting the BRAF V600E mutation in multiple myeloma. Cancer Discov 2013;3:862–9.
36. Heuck CJ, Jethava Y, Khan R, van Rhee F, Zangari M, Chavan S, et al. Inhibiting MEK in MAPK pathway-activated myeloma. Leukemia 2016;30: 976–80.
37. Cottini F, Hideshima T, Xu C, Sattler M, Dori M, Agnelli L, et al. Rescue of YAP1 triggers DNA damage-induced apoptosis in hematological cancers. Nat Med 2014;20:599–606.
38. Cottini F, Hideshima T, Suzuki R, Tai Y-T, Bianchi G, Richardson PG, et al. Synthetic lethal approaches exploiting DNA damage in aggressive myeloma. Cancer Discov 2015;5:972–87.
39. Ohguchi H, Hideshima T, Bhasin MK, Gorgun GT, Santo L, Cea M, et al. The KDM3A-KLF2-IRF4 axis maintains myeloma cell survival. Nat Commun 2016;7:10258.
40. Richardson PG, Barlogie B, Berenson J, Singhal S, Jagannath S, Irwin D, et al. A multicenter phase II study of bortezomib in patients with relapsed and refractory multiple myeloma. N Engl J Med 2003;348:2609–17.
41. Stewart AK, Rajkumar SV, Dimopoulos MA, Masszi T, Spicka I, Oriol A, et al. Carfilzomib, lenalidomide, and dexamethasone for relapsed multiple myeloma. N Engl J Med 2015;372:142–52.
42. Moreau P, Masszi T, Grzasko N, Bahlis NJ, Hansson M, Pour L, et al. Oral ixazomib, lenalidomide, and dexamethasone for multiple myeloma. N Engl J Med 2016;374:1621–34.
43. Richardson PG, Zimmerman TM, Hofmeister CC, Talpaz M, Chanan-Khan A, Kaufman JL, et al. Phase 1 study of marizomib in relapsed or relapsed and refractory multiplte myeloma: NPI0052 Part 1. Blood 2016;127: 2693–700.
44. Dimopoulos M, Spencer A, Attal M, Prince HM, Harousseau JL, Dmoszynska A, et al. Lenalidomide plus dexamethasone for relapsed or refractory multiple myeloma. N Engl J Med 2007;357:2123–32.
45. McCarthy PL, Owzar K, Hofmeister CC, Hurd DD, Hassoun H, Richardson PG, et al. A phase III study of lenalidomide after transplant for multiple myeloma. N Engl J Med 2012;366:1770–81.
46. Benboubker L, Dimopoulos MA, Dispenzieri A, Catalano J, Belch AR, Cavo M, et al. Lenalidomide and dexamethasone in transplant-ineligible patients with myeloma. N Engl J Med 2014;371:906–17.
47. San Miguel J, Weisel K, Moreau P, Lacy M, Song K, Delforge M, et al. Pomalidomide plus low-dose dexamethasone versus high-dose dexamethasone alone for patients with relapsed and refractory multiple myeloma (MM-003): a randomised, open-label, phase 3 trial. Lancet Oncol 2013;14: 1055–66.
48. Hideshima T, Anderson KC. Molecular mechanisms of novel therapeutic approaches for multiple myeloma. Nat Rev Cancer 2002;2: 927–37.
49. Hideshima T, Mitsiades C, Tonon G, Richardson P, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 2007;7:585–98.
50. Mitsiades CS, Mitsiades N, Bronson RT, Chauhan D, Munshi N, Treon SP, et al. Fluorescence imaging of multiple myeloma cells in a clinically-relevant SCID/NOD in vivo model: biologic and clinical implications. Cancer Res 2003;63:6689–96.
51. Tassone P, Neri P, Burger R, Carrasco DR, Goldmacher VS, Fram R, et al. A clinically relelvant SCID-hu *in vivo* model of human multiple myeloma. Blood 2005;106:713–6.
52. Urashima M, Chen BP, Chen S, Pinkus GS, Bronson RT, Dedera DA, et al. The development of a model for the homing of multiple myeloma cells to human bone marrow. Blood 1997;90:754–65.
53. McMillin DW, Delmore J, Weisberg E, Negri JM, Geer DC, Klippel S, et al. Tumor cell-specific bioluminescence platform to identify stroma-induced changes to anti-cancer drug activity. Nat Med 2010;16:483–9.
54. Tai YT, Dillon M, Song W, Leiba M, Li XF, Burger P, et al. Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody dependent cellular cytotoxicity in the bone marrow milieu. Blood 2008;112:1329–37.
55. de Weers M, Tai Y-T, van der Veer MS, Bakker JM, Vink T, Jacobs DCH, et al. Daratumumab, a novel therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. J Immunol 2011;186:1840–8.
56. Tai Y-T, Mayes PA, Acharya C, Zhong MY, Cea M, Cagnetta A, et al. Novel afucosylated anti-B cell maturation antigen-monomethyl auristatin F antibody-drug conjugate (GSK2857916) induces potent and selective anti-multiple myeloma activity via direct tumor cell killing and enhanced effector function. Blood 2014;123:3128–38.
57. Mimura N, Hideshima T, Suzuki R, Ohguchi H, Cottini F, Tai Y-T, et al. Selective and potent Akt inhibition triggers anti-myeloma activities and enhances fatal endoplasmic reticulum stress induced by proteasome inhibition. Cancer Res 2014;74:4458–69.
58. Zhu D, Wang Z, Zhao J-J, Calimeri T, Meng J, Hideshima T, et al. The cyclophilinA-CD147 complex promotes bone marrow colonization of B-cell malignancies: implications for therapy. Nat Med 2015;21:572–80.
59. Tai Y-T, Acharya C, An G, Moschetta M, Zhong MY, Feng X, et al. April and BCMA promote human multiple myeloma growth, chemoresistance, and immunosuppression in the bone marrow microenvironment. Blood 2016;127:3225–36.
60. Hideshima T, Mazitschek R, Santo L, Mimura N, Gorgun G, Richardson PG, et al. Induction of differential apoptotic pathways in multiple myeloma cells by class selective histone deacetylase inhibitors. Leukemia 2014;28: 457–60.
61. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutc strategy to target c-Myc. Cell 2011;146:904–17.
62. Mitsiades CS, Mitsiades N, McMullan CJ, Poulaki V, Shringarpure R, Akiyama M, et al. Inhibition of the insulin-like growth factor receptor-1 tyrosine kinase activity as a therapeutic strategy for mutliple myeloma, other hematologic malignancies and solid tumors. Cancer Cell 2004;5:221–30.
63. Chauhan D, Singh AV, Brahmandam M, Carrasco R, Bandi M, Podar K, et al. Functional interaction of plasmacytoid dendritic cells with multiple myeloma cells: a novel therapeutic target. Cancer Cell 2009;16:1–15.
64. Gorgun G, Whitehill G, Anderson JL, Hideshima T, Maguire CJ, Laubach J, et al. Tumor promoting immune suppressive myeloid derived suppressor cells in multiple myeloma microenvironment. Blood 2012; 121:2975–87.
65. Ray A, Das DS, Song Y, Richardson P, Chauhan D, Anderson KC. Targeting PD1-PDL1 in immune checkpoint in plasmacytoid dendritic cell interactions with T cells, natural killer cells, and multiple myeloma cells. Leukemia 2015;29:1441–4.
66. An G, Acharya C, Feng X, Wen K, Zhong M, Zhong M, et al. Osteoclasts promote immune suppressive microenvironment in multiple myeloma. Blood 2016;128:1590–603.
67. Orlowski RZ, Lonial S. Integration of novel agents into the care of patients with multiple myeloma. Clin Cancer Res 2016;22:5443–52.

68. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J, et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis and overcomes drug resistance in human multiple myeloma cells. Cancer Res 2001;61:3071–6.
69. Mitsiades N, Mitsiades CS, Poulaki V, Chauhan D, Gu X, Bailey C, et al. Molecular sequelae of proteasome inhibition in human multiple myeloma cells. Proc Natl Acad Sci U S A 2002;99:14374–9.
70. Hideshima T, Mitsiades C, Akiyama M, Hayashi T, Chauhan D, Richardson P, et al. Molecular mechanisms mediating anti-myeloma activity of proteasome inhibitor PS-341. Blood 2003;101:1530–4.
71. LeBlanc R, Catley LP, Hideshima T, Lentzsch S, Mitsiades CS, Mitsiades N, et al. Proteasome inhibitor PS-341 inhibits human myeloma cell growth in vivo and prolongs survival in a murine model. Cancer Res 2002; 62:4996–5000.
72. Richardson PG, Sonneveld P, Schuster MW, Irwin D, Stadtmauer EA, Facon T, et al. A randomized comparison of Bortezomib with high-dose dexamethasone in relapsed multiple myeloma. N Engl J Med 2005;352:2487–98.
73. San Miguel JF, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, Kropff M, et al. Bortezomib plus melphalan-prednisone versus melphalan-prednisone in untreated multiple myeloma patients ineligible for stem cell transplantation. N Engl J Med 2008;359:906–17.
74. Chauhan D, Tian Z, Zou B, Raje N, Richardson PG, Anderson KC. *In vitro* and *in vivo* selective antitumor activity of a novel orally bioavailable proteasome inhibitor MLN9708 against multiple myeloma cells. Clin Cancer Res 2011;17:5311–21.
75. Tian Z, Zhao JJ, Tai YT, Amin SB, Hu Y, Berger AJ, et al. Investigational agent MLN9708/2238 targets tumor suppressor microRNA-33b in MM cells. Blood 2012;120:3958–67.
76. Kumar SK, Berdeja JG, Niesvizky R, Lonial S, Laubach JP, Hamadani M, et al. Safety and tolerability of ixazomib, an oral proteasome inhibitor, in combination with lenalidomide and dexamethasone in patients with previously untreated multiple myeloma: an open-label phase 1/2 study. Lancet Oncol 2014;15:1503–12.
77. Chauhan D, Catley L, Li G, Podar K, Hideshima T, Velankar M, et al. A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from bortezomib. Cancer Cell 2005;8: 407–19.
78. Chauhan D, Tian Z, Nicolson B, Zhou B, Kumar KGS, Carrasco R, et al. A novel small molecule inhibitor of ubiquitin-specific protease-7 induces apoptosis in multiple myeloma cells and overcomes bortezomib resistance. Cancer Cell 2012;22:345–58.
79. Tian Ze, D'Arcy P, Wang X, Ray A, Tai Y-T, Hu Y, et al. A novel small molecule inhibitor of deubiquitylating enzyme USP14 and UCHL5 induces apoptosis in myeloma cells and overcomes bortezomib resistance. Blood 2014;123:706–16.
80. Song Y, Ray A, Li S, Das D, Tai YT, Carrasco RD, et al. Targeting proteasome ubiquitin receptor Rpn13 in multiple myeloma. Leukemia 2016;30: 1877–86.
81. Hideshima T, Bradner J, Wong J, Chauhan D, Richardson P, Schreiber S, et al. Small molecule inhibition of proteasome and aggresome function induces synergistic anti-tumor activity in multiple myeloma. Proc Natl Acad Sci U S A 2005;102:8567–72.
82. Santo L, Hideshima T, Kung AL, Tseng JC, Tamang D, Yang M, et al. Preclinical activity, pharmacodynamic, and pharmacokinetic properties of a selective HDAC6 inhibitor, ACY-1215, in combination with bortezomib in multiple myeloma. Blood 2012;119:2579–89.
83. Lu G, Middleton RE, Sun H, Naniong M, Ott CJ, Mitsiades CS, et al. Kaelin WG JrThe myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. Science 2014;343:305–9.
84. Krönke J, Udeshi ND, Narla A, Grauman P, Hurst SN, McConkey M, et al. Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells. Science 2014;343:301–5.
85. Winter GE, Buckley DL, Paulk J, Roberts JM, Souza A, Dhe-Paganon S, et al. DRUG DEVELOPMENT. Phthalimide conjugation as a strategy for *in vivo* target protein degradation. Science. 2015;348:1376–81.
86. Kumar SK, Anderson KC. Immune therapies in myeloma. Clin Cancer Res 2016;22:5453–60.
87. Hideshima T, Chauhan D, Shima Y, Raje N, Davies FE, Tai YT, et al. Thalidomide and its analogues overcome drug resistance of human multiple myeloma cells to conventional therapy. Blood 2000;96:2943–50.
88. Davies FE, Raje N, Hideshima T, Lentzsch S, Young G, Tai YT, et al. Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. Blood 2001;98:210–6.
89. Gorgun G, Calabrese E, Soydan E, Hideshima T, Perrone G, Bandi M, et al. Immunomodulatory effects of lenalidomide and pomalidmide on interaction of tumor and bone marrow accessory cells in multiple myeloma. Blood 2010;116:3227–37.
90. Gorgun G, Samur MK, Cowens KB, Paula S, Bianchi G, Anderson JE, et al. Lenalidomide enhances immune checkpoint blockade induced immune response in multiple myeloma. Clin Cancer Res 2015;21: 4607–18.
91. Rosenblatt J, Avivi I, Vasir B, Uhl L, Munshi NC, Katz T, et al. Vaccination with dendritic cell/tumor fusions following autologous stem cell transplant induces immunologic and clinical responses in multiple myeloma patients. Clin Cancer Res 2013;19:3640–8.
92. Ali SA, Shi V, Maric I, Wang M, Stroncek DF, Rose JJ, et al. T cells expressing an anti-B-cell-maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. Blood 2016;128:1688–700.
93. Tai YT, Anderson KC. Antibody-based therapies in multiple myeloma. Bone Marrow Res 2011;2011:9204058.

Clinical Cancer Research

Progress and Paradigms in Multiple Myeloma

Kenneth C. Anderson

Clin Cancer Res 2016;22:5419-5427.