Novel Targeted Agents in the Treatment of Multiple Myeloma

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KEYWORDS

- Multiple myeloma • Second-generation proteasome inhibitors
- Histone deacetylase inhibitors • Heat shock protein 90 inhibitors
- PIK3/Akt/mTOR inhibitors • BET bromodomain inhibitors
- Deubiquitinating enzyme inhibitors • Wnt

KEY POINTS

- New, next-generation targeted treatment strategies are urgently required to improve outcomes in patients with multiple myeloma (MM).
- Monoclonal antibodies, cell signaling inhibitors, and selective therapies targeting the bone marrow microenvironment have demonstrated encouraging results with generally manageable toxicity in therapeutic trials of patients with relapsed and refractory disease, each critically informed by preclinical studies.
- A combination approach of these newer agents with immunomodulators and/or proteasome inhibitors as part of a treatment platform seems to consistently improve the efficacy of anti-MM regimens, even in heavily pretreated patients.
- Future studies continue to be required to better understand the complex mechanisms of drug resistance in MM.
- Incorporating molecular correlates to further personalize treatment and to, thus, better integrate these agents into clinical practice is a clear priority.

INTRODUCTION

Multiple myeloma (MM) is the second most common hematologic malignancy after non-Hodgkin lymphoma and remains incurable despite major advances in therapy over the last decade, with the use of bortezomib, thalidomide, and lenalidomide as

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first-generation novel therapies in particular impacting favorably on prognosis. MM remains challenging because of both tumor-specific factors, including adverse mutations that result in both inherent and acquired resistance to therapy, and enhanced tumor survival derived from the surrounding bone marrow microenvironment. Newer targeted treatment strategies are currently under development and show considerable promise in overcoming this resistance; these include second-generation proteasome inhibitors (PIs) (such as carfilzomib), third-generation immunomodulators (specifically pomalidomide), monoclonal antibodies in particular, and other cell signaling inhibitors, as well as specific therapies targeting the bone marrow microenvironment. Some of these agents already have proven highly efficacious in the relapsed and refractory (RR) setting, specifically carfilzomib and pomalidomide; this leads to their recent regulatory approval. This review focuses on novel targeted therapies currently under investigation and in various stages of clinical trials, with approvals pending and/or breakthrough designation assigned to several agents.

SECOND-GENERATION PROTEOSOME INHIBITORS

Ixazomib (MLN9708)

MLN9708 is a dipeptidilic boronic acid that immediately hydrolyzes to MLN2238, an active form, on exposure to aqueous solutions. MLN2238 reversibly and selectively inhibits the 20S proteasome. It has a 6-fold faster dissociation half-life and greater tissue penetration as compared with bortezomib (**Fig. 1**).[1] In a xenograft model, MLN2238 showed significantly longer survival in tumor-bearing mice treated with MLN2238 than mice receiving bortezomib. MLN2238 was found to be active even in bortezomib-resistant cells.[2] MLN2238 can synergize with lenalidomide, vorinostat, and/or dexamethasone in combination.[2] Importantly, MLN9708 did not demonstrate significant inhibition of neuronal cell survival, which may explain the lack of major peripheral neuropathy (PN) seen so far with this PI.[2]

MLN9708 was the first oral PI to be incorporated into clinical trials. Several phase I studies have evaluated the safety of MLN9708 using both oral and intravenous (IV) routes of administration. Data from these studies have demonstrated linear pharmacokinetics, regardless of administration route.[3]

Two phase I studies, one using weekly dosing[4] and the other using biweekly dosing,[5] have evaluated the oral administration of MLN9708 as a single agent in heavily pretreated patients with RR MM previously exposed to PIs. In the 41 evaluable patients receiving weekly dosing, responses included 1 very good partial response (VGPR), 5 partial responses (PR), 1 minimal response (MR), and 15 with stable disease (SD).[4] In the 46 evaluable patients receiving biweekly dosing, 6 patients achieved PR or more, including 1 stringent complete remission (sCR) and 5 PR.[5]

The most common all-grades adverse events (AEs) included fatigue (30%–40%), thrombocytopenia (30%–40%), nausea (30%), diarrhea (25%), vomiting (20%), as well as rash and neutropenia. Drug-related PN was minimal at 4% to 8%, and none were grade 3 or more. Toxicities proved generally manageable with dose reduction and supportive care, and tolerability overall was considered favorable.

Preliminary data from phase I/II studies of once-weekly and biweekly oral MLN9708 in combination with lenalidomide and dexamethasone in patients with newly diagnosed myeloma were recently reported. Drug-related AEs were similar to the phase I studies, although more frequent with twice-weekly administration. Among evaluable patients who received biweekly dosing, 95% achieved PR or better, with a 27% CR/sCR rate. The depth of response increased over the course of treatment. The median time to best response was 1.96 months.[6] MLN9708 in combination with lenalidomide

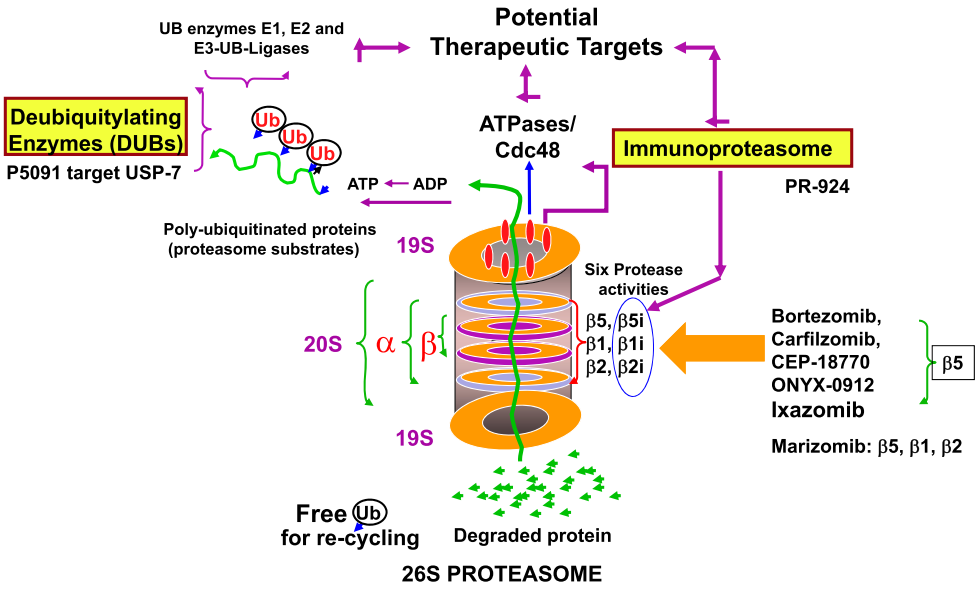

Fig. 1. Proteasome: present and future therapies. UB, ubiquitin. (*Adapted from* Lawasut P, Chauhan D, Laubach J, et al. New proteasome inhibitors in myeloma. Curr Hematol Malig Rep 2012;7:258–66, with permission; and *Data from* Refs.[9,13,60,127,128])

and dexamethasone is currently being investigated in a phase III trial in newly diagnosed patients as well as having been recently completed in patients with relapsed disease, with results expected next year. The benefit of maintenance therapy with this agent is also being evaluated both as a single agent and in combination with lenalidomide.

Oprozomib (ONX 0912)

Oprozomib (ONX 0192) is an epoxyketone PI and is an orally available analogue of carfilzomib. Both carfilzomib and ONX 0912 are irreversible PIs that selectively inhibit the chymotrypsin-like (CT-L) subunit (β5 and β5i) and, thus, have minimal off-target effects (see **Fig. 1**).[7–9] This PI has shown a significant anti-MM response in vitro and in vivo studies.[9,10] ONX 0912 is currently being evaluated in phase I and II dose-escalating studies.[11] Reported adverse effects are mainly gastrointestinal and similar to observations in preclinical animal models.[12] Although promising activity has been seen, serious gastrointestinal toxicity has been reported; concerted efforts to improve tolerability are underway.

NPI-0052 (Marizomib)

NPI-0052 (Marizomib) is part of a unique class of PIs, in that it is a natural β-lactone compound that can irreversibly inhibit CT-L, trypsin-like (T-L), and caspase-like (C-L) proteasomal activities in vitro and in vivo (see **Fig. 1**).[13] Through its covalent binding of all 3β subunits, NPI-0052 demonstrates comparable or even greater proteasome inhibition compared with bortezomib and carfilzomib in preclinical models.[13] NPI-0052 is also highly synergistic with either lenalidomide or pomalidomide in vitro and overcomes bortezomib resistance preclinically.[14,15]

Subsequent results from a dose-escalating study in patients with RR MM with twice-weekly IV NPI-0052 has been reported,[16] with 20% (3 of 15) of bortezomib-resistant patients achieving PR, and 73% of all evaluable patients (n = 22) achieving at least SD. Reported dose-limiting toxicities (DLTs) included predominantly mild to moderate hallucinations, cognitive changes, and loss of balance, which were transient and reversible with dose reduction. Importantly, there was no evidence of treatment-emergent PN or significant myelosuppression. A twice-weekly regimen is currently being investigated, and combination studies with pomalidomide and dexamethasone are underway.

CELL SIGNALING AGENTS

Histone Deacetylases Inhibitors

Epigenetic modification plays a diverse role in both physiologic and pathologic cellular processes. Acetylation, one of the most frequent alterations in epigenetics, serves as a key player in the regulation of gene expression by altering chromatin structure without modifying the underlying DNA.[17,18] Acetylation is tightly regulated by 2 opposing enzymes: histone deacetylases (HDACs) and histone acetyltransferases (HATs). Although hyperacetylation of the histone NH2 tail by HATs results in open chromatin and gene expression, HDACs have shown to have a repressive effect on transcription by mediating a closed chromatin conformation.[19,20] Transcriptional repression by HDACs is implicated in carcinogenesis,[21] making them a promising therapeutic target. HDACs also act on many other nonhistone substrate proteins involved in the modulation of transcription.[22,23]

HDAC inhibitors bind to the catalytic sites of HDACs, preventing accessibility of transcription factors to promoter regions[22,23] and also upregulate negative cell cycle

modulators of the G1 phase, such as $p21^{WAF1}$ and $p27^{Kip1}$.[24,25] HDAC inhibitors can be categorized as class–specific inhibitors or as pan-deacetylase (pan-DAC) inhibitors, the latter denoting activity against both class I and II recombinant HDACs.[26]

Panobinostat

Panobinostat is a potent pan-DAC inhibitor, which has potent inhibitory activity at low nanomolar concentrations against all class I, II, and IV HDACs (**Fig. 2**).[27] Panobinostat leads to the acetylation of lysine residues across intracellular targets.[28] In preclinical studies, panobinostat had an antimyeloma effect using in vitro and in vivo myeloma models.[28–30] Based on this data, a phase II study of oral panobinostat as monotherapy in heavily pretreated patients with RR MM was investigated.[31] Patients had at least 2 prior treatments, including bortezomib, thalidomide, and lenalidomide. Panobinostat was administered at a dosage of 20 mg 3 times weekly out of a 21-day treatment cycle. Overall, one PR and one MR were observed out of 38 evaluable patients; however, these responses were maintained for 19 and 28 months, respectively.[31] All grade AEs reported included gastrointestinal toxicity (80.0%) and hematologic effects, which included grade 3/4 neutropenia (31.6%), thrombocytopenia (26.3%), and anemia (18.4%).

Panobinostat was next evaluated in combination with bortezomib in patients with RR MM, based on preclinical studies, which demonstrated synergy.[29,30,32] Specifically, in a phase Ib study of panobinostat and bortezomib,[33] clinical responses were observed (including complete responses) in patients with bortezomib-refractory MM; toxicity proved manageable.

PANORAMA 2 was a multicenter phase II study examining the combination of oral panobinostat with bortezomib and dexamethasone in 55 heavily pretreated patients

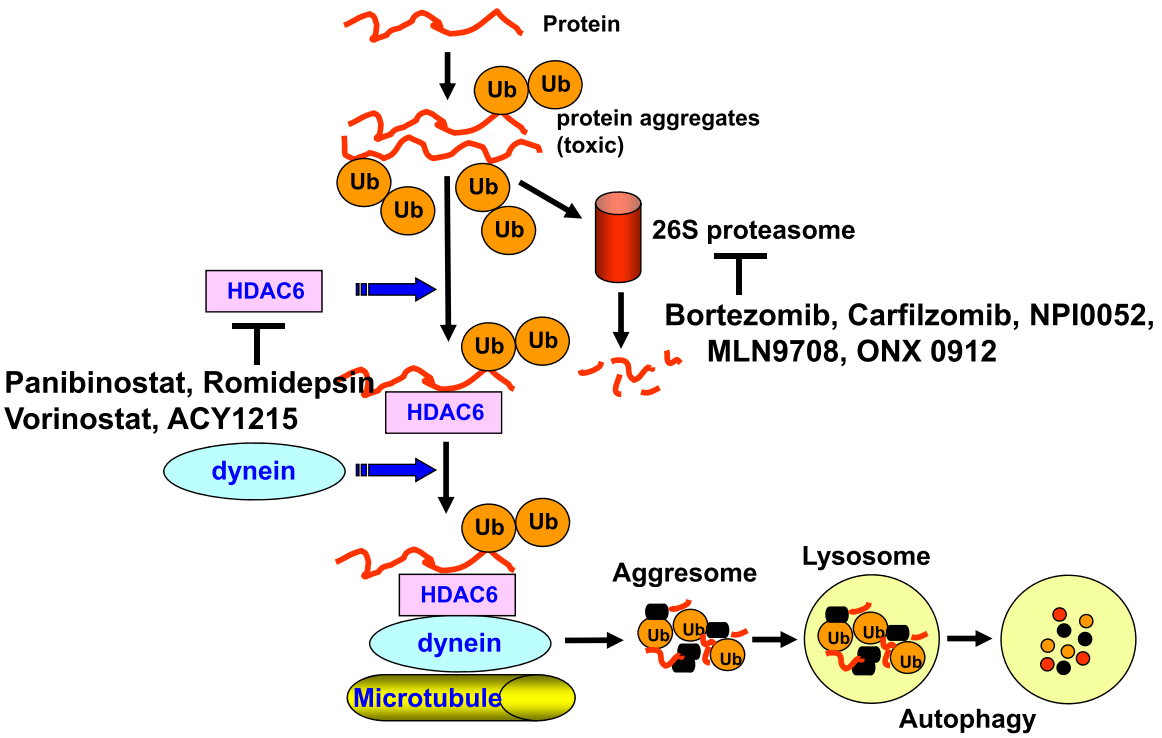

Fig. 2. Development of rationally based combination therapies (HDAC and PIs). (*Adapted from* Hideshima T, Bradner JE, Chauhan D, et al. Intracellular protein degradation and its therapeutic implications. Clin Cancer Res 2005;11:8530, with permission; and Catley L, Weisberg E, Kiziltepe T, et al. Aggresome induction by proteasome inhibitor bortezomib and alpha-tubulin hyperacetylation by tubulin deacetylase (TDAC) inhibitor LBH589 are synergistic in myeloma cells. Blood 2006;108(10):3441–9.)

with relapsed and bortezomib-refractory MM.[34] The overall response rate (ORR) was 34.5% (including 1 near PR and 18 PRs), with 10 patients achieving an MR, resulting in a clinical benefit rate (CBR) of 52.7%. Among patients with adverse cytogenetics (n = 14), the ORR was 43% and the CBR was 71%. The median progression-free survival (PFS) was 5.4 months. Common grade 3/4 AEs included thrombocytopenia (63.6%), fatigue (20.0%), and diarrhea (20.0%).[34]

Based on these encouraging data, a phase III clinical trial was performed. PANORAMA 1 was an international, randomized, double-blinded study of panobinostat (vs placebo) in combination with bortezomib and dexamethasone in patients with RR MM. A total of 768 patients were randomized. Preliminary results demonstrated an ORR of 61% in the panobinostat arm versus 55% in the placebo arm, with an near CR (nCR)/CR rate of 28% versus 16% ($P = .00006$), respectively. The median PFS was 12.0 months versus 8.1 months ($P<.0001$; hazard ratio 0.63, 95% confidence interval [0.52, 0.76]) in favor of the panobinostat arm. Common grade 3/4 AEs in the panobinostat versus placebo arms included thrombocytopenia (67% vs 31%), neutropenia (35% vs 11%), and diarrhea (26% vs 8%), which were generally manageable with dose reduction and/or supportive care.[35] Given the PFS benefit of 4 months in favor of panobinostat and its activity in high-risk groups in particular, as well as the encouraging quality of response differences seen, regulatory approval is anticipated this year.

Vorinostat

Similar to panobinostat, vorinostat is also a pan-DAC inhibitor in the hydroxamic class (see **Fig. 2**).[36] Mitsiades and colleagues demonstrated in vitro antimyeloma activity when MM cells were irreversibly committed to cell death after hours of incubation with vorinostat. Using microarray analyses, vorinostat-induced apoptosis was associated with suppression of genes mediating cytokine-driven proliferation and survival, drug-resistance, DNA synthesis/repair, and proteasome function.[37] In MM cell lines, vorinostat successfully induced apoptosis in all tumor cells with increased levels of proapoptotic protein levels of p21 and p53. Vorinostat also inhibited the secretion of interleukin 6 (IL-6) produced by bone marrow stromal cells (BMSCs), suggesting that HDAC inhibitors can overcome cell adhesion–mediated drug resistance (CAM-DR).[38] An ongoing phase I trial examined the combination of vorinostat with lenalidomide, bortezomib, and dexamethasone in patients with newly diagnosed MM. Vorinostat was administered orally at 100, 200, 300, or 400 mg daily on days 1 to 14 of each cycle. Thirty patients were enrolled with an ORR (PR or better) of 100%, with a VGPR or better of 52%, and a CR rate of 28%. At a median follow-up of 11.5 months (range 1–31 months), there has been only one patient with progressive disease.[39] Similarly encouraging results with vorinostat in combination with lenalidomide in RR MM were seen in a phase I combination study with an ORR of 47% and favorable tolerability.[40] In contrast, efforts with vorinostat combined with bortezomib proved challenging; ultimately, the prospective international randomized phase III trial of vorinostat combined with bortezomib versus bortezomib alone failed to demonstrate a meaningful clinical benefit. This finding was despite significantly higher rates of response in favor of the combination and largely because of the excessive toxicity seen with the particular dose and schedule of vorinostat used, as well as the absence of dexamethasone use, resulting in only a minimal PFS improvement of less than a month seen between the doublet and the monotherapy control.[41]

ACY-1215 (Rocilinostat)

HDAC6 plays an important role in the breakdown of ubiquitinated proteins and in the formation of perinuclear aggresomes. Blocking HDAC6 activity results in the

accumulation of polyubiquitinated proteins, which, in turn, induces cell stress followed by apoptosis.[42] HDAC6 inhibition markedly enhances the action of PIs, making HDAC6 a promising novel target for this approach as well as with other combinations. Furthermore, the more selective inhibition of HDAC6 may reduce the off-target toxicity previously seen with pan-HDAC inhibitors.[42,43]

ACY-1215 is a novel, selective HDAC6 inhibitor that is orally available (see **Fig. 2**). Santo and colleagues[44] evaluated the action of ACY-1215 alone and in combination with bortezomib in the preclinical setting. In this study, the combination of both proteasome and HDAC6 inhibition lead to synergistic cytotoxicity, resulting in apoptosis of MM cells by activation of the caspase pathway. In vivo experiments using 2 xenograft severe combined immunodeficiency (SCID) mouse models, confirmed the anti-MM effects of ACY-1215 when combined with bortezomib. The mouse treated with both agents experienced a significantly prolonged overall survival and delayed tumor growth.[44] This study prompted the rationale to use ACY-1215 in clinical trials.

ACY-100 is a single arm, open-label, dose-escalation trial using ACY-1215 in patients with RR MM as monotherapy (phase Ia) and in combination with bortezomib (phase Ib) followed by a phase II extension. ACY-1215 was given orally on days 1 to 5 and 8 to 12 of a 21-day cycle. Most AEs were grade 1 to 2, whereas 2 patients had grade 3 AEs (anemia and neutropenia). No DLTs were observed. Six patients had SD as their best response.[45]

In the combination cohort, treatment-related AEs were mainly low grade, with the majority not considered related to ACY-1215. The first cohort was expanded because of a DLT of asymptomatic increase in amylase, but no other DLTs have been observed. Grade 3 to 4 AEs included asymptomatic elevated amylase, thrombocytopenia, anemia, stomach cramps, and an increase in creatinine. Of the 16 evaluable patients, 1 VGPR, 2 PR, 1 MR, and 5 SD were reported. Of the patients who were previously refractory to bortezomib, the best outcome at the time of presentation was 1 VGPR, 1 MR, and 4 SD,[45] with recent updates suggesting greater ORR with more time on therapy.

Based on synergy observed between ACY-1215 and lenalidomide in preclinical studies, a phase I trial investigating this combination treatment regimen in patients with RR MM is being carried out. In part A, patients were treated with escalating doses of oral ACY-1215 on days 1 to 5 and 8 to 12 of a 28-day cycle, with lenalidomide 25 mg on day 1 to 21 and dexamethasone 40 mg weekly.[46] Most treatment-related events were low grade and included fatigue (43%), upper respiratory infection (36%), anemia and peripheral edema (21% each), neutropenia (29%) and muscle spasms (21%). There were 9 grade 3 to 4 events in 6 patients, which were primarily hematologic. Nine patients (69%) achieved responses of PR or greater, including 1 CR, 4 VGPR, and 3 PR. Of the 6 patients who were previously refractory to lenalidomide, the best responses included 1 PR, 1 VGPR, 2 MR, and 2 SD.[46] Future studies are now evaluating the combination of ACY-1215 with pomalidomide and with whom even greater preclinical activity is seen; the all-oral 3-drug platform of AC1215, pomalidomide, and dexamethasone is, therefore, hoped to be a particularly important triplet going forward.

Heat-Shock Protein 90 Inhibitors

Heat-shock protein 90 (Hsp90) regulates cellular trafficking by facilitating the 3-dimensional folding of intracellular proteins implicated in cell proliferation and drug resistance.[47] In tumors, Hsp90s are an important target, as they act as a chaperone to mutated or overexpressed proteins that promote cell survival. In a phase I/II clinical trial, patients with RR MM were administered the hsp90 inhibitor tanespimycin

(100–340 mg/m^2) and bortezomib (0.7–1.3 mg/m^2 IV) on days 1, 4, 8, and 11 in each 21-day cycle. Among the 67 evaluable patients, there were 2 (3%) complete responses and 8 (12%) PRs, for an ORR of 27%, including 8 (12%) MRs.[48] The most common AEs were diarrhea (60%), nausea (49%), fatigue (49%), thrombocytopenia (40%), transient elevations in AST (28%) and dizziness (28%). Most toxicities were grade 1 or grade 2. There was no reported grade 3/4 peripheral neuropathy.[48] Unfortunately, the tanespimycin program had to be closed prematurely because of insurmountable difficulties in producing adequate and high-quality amounts of the drug substance. Other studies of Hsp90 inhibition are ongoing, and early results show some promise (eg, AUY922).

Phosphoinosiide 3-kinase/Akt/Mammalian Target of Rapamycin Pathway Inhibitors

Akt modulates the phosphorylation of several downstream substrates involved in cellular growth and survival.[49] One of the most studied downstream protein kinases is the mammalian target of rapamycin, which has been implicated in the pathogenesis of several different cancers. In MM, the phosphoinosiide 3-kinase (PI3K)/Akt pathway is overactive, thus inhibiting apoptosis and allowing for clonal cell expansion. Hsu and colleagues[50] demonstrated, using immunohistochemistry, that Akt is frequently activated in MM cells and the frequency is directly proportional to the disease stage.[50] Interruption of the Akt pathway resulted in inhibition of MM cell growth in vitro.

Perifosine is a biologically available alkylphospholipid that inhibits the Akt pathway and, thus, promotes apoptosis in MM cells. In preclinical studies, baseline phosphorylation of Akt in MM cells was completely inhibited by perifosine in a time- and dose-dependent manner. Perifosine was also successful in inducing apoptosis even in MM cells adherent to BMSCs.[51] Perifosine was found to enhance the cytotoxic effects of novel agents, such as bortezomib.[51] Taken together, these data provided the rationale for clinical trials using Akt pathway inhibitors in the setting of patients with RR MM.

A phase I multicenter single-arm study was carried out looking at escalating doses of perifosine 50 to 100 mg in combination with lenalidomide plus dexamethasone 20 to 40 mg weekly. The most common AEs were grade 1 to 2 fatigue (48%) and diarrhea (45%), and grade 3 to 4 neutropenia (26%), hypophosphatemia (23%), thrombocytopenia (16%), and leucopenia (13%). MR or better was attained in 73% of evaluable patients, including 50% with a PR or better.

In a multicenter phase I/II study, perifosine was combined with bortezomib with or without dexamethasone in 84 patients with RR MM.[52] All patients were heavily pretreated, and many were resistant to bortezomib. The ORR (MR or better) was 41%, including an ORR of 32% in bortezomib-refractory patients. The median PFS was 6.4 months, with a median overall survival of 25 months. Treatment was well tolerated. Common treatment-related grade 1 and 2 AEs included nausea (63%), diarrhea (57%), fatigue (43%), musculoskeletal pain (42%), anorexia, and upper respiratory tract infections (33% each). All AEs were manageable with supportive care and dose reductions. Grade 3 or more toxicities included thrombocytopenia (23%), neutropenia (15%), anemia (14%), and pneumonia (12%).[52] Unfortunately, the pivotal prospective phase III study of perifosine, bortezomib, and dexamethasone versus placebo, bortezomib, and dexamethasone was closed prematurely as a result of resource constraints, slow accrual, and equivocal findings at interim analysis. Other studies with more potent Akt inhibitors are showing considerable promise (eg, GSK2110183). Most recently, Mimura and colleagues[53] also demonstrated anti-MM activities of a novel allosteric inhibitor TAS-117 alone and in combination with bortezomib.

BET Bromodomain Inhibitors

Myc plays a key role in the pathogenesis of many human cancers, including MM.[54] We have yet to discover therapeutic approaches to modulate the function of the c-Myc oncoprotein. Bromodomains are important recognition domains of coactivator proteins implicated in the initiation of transcription. Disruption of the bromodomains will interfere with signal transduction and, ultimately, will inhibit the transcription of the Myc oncoprotein. JQ1 is a selective small-molecule BET bromodomain inhibitor that downregulates Myc transcription and the expression of other Myc-dependent target genes.[55]

Antiproliferative activity of JQ1 was assessed using in vitro and xenograft models. MM cell proliferation was uniformly inhibited by JQ1. These samples included several cell lines resistant to Food and Drug Administration–approved agents. In primary cells isolated from a patient with RR MM, JQ1 treatment resulted in a time-dependent suppression of c-Myc expression.[55] It is hoped that clinical efficacy of the JQ1 inhibitor will be confirmed in human studies; clinical trials are now underway, with combination studies incorporating lenalidomide due to commence this year.

Deubiquitinating Enzyme Inhibitors

Ubiquitin regulates the degradation of proteins via proteasomes and lysosomes and modulates protein-protein interactions.[56,57] Deubiquitinating enzymes (DUBs) are a group of proteases that cleave the bond between ubiquitin and its substrate protein (see **Fig. 1**).[58] Inhibition of DUBs or proteasome results in the accumulation of ubiquitinated proteins.[59] The novel regulatory particle b-AP15 selectively blocks deubiquitinating activity without inhibiting proteasome activity. In preclinical studies, b-AP15 was shown to decrease viability in bortezomib-resistant MM cell lines and patient MM cells, even in the presence of BMSCs[60]; b-AP15 demonstrated good tolerability in human MM xenograft models. Combining b-AP15 with lenalidomide or dexamethasone induced synergistic anti-MM activity. DUB inhibitors will need to be further investigated as potential therapeutic agents to improve clinical outcomes in MM. To this end, clinical studies are planned and are expected to begin shortly.

Wnt, Hedgehog, Notch Inhibitors

Wnt

Wnt proteins are glycoproteins that serve as ligands to transmembrane receptors. Abnormal Wnt signaling has been described in MM.[61] Dickkopf 1 (DKK1) is a soluble antagonist of the Wnt pathway that is overexpressed by plasma cells in patients with osteolytic lesions. The overexpression of DKK1 blocks the differentiation of osteoblasts and, thus, inhibits the formation of bone.[62] BHQ880 is a fully human neutralizing antibody targeting DKK1. Preclinical studies have demonstrated that BHQ880 reduces IL-6 secretion and promotes osteoblastogenesis in vitro and in mouse models.[63] Preliminary data from a phase I study in patients with RR MM receiving both zoledronic acid and BHQ880 demonstrated an increase in bone density in some patients.[64] An open-label, multicenter, single-arm, phase II study designed to evaluate the safety and antimyeloma activity of BHQ880 in patients with high-risk smoldering MM (SMM) has recently been completed with excellent tolerability and some suggestion of activity seen.[65]

Notch

The Notch pathway regulates cell proliferation, cellular differentiation, and programmed cell death. It is implicated in the pathophysiology of multiple hematologic malignancies, including MM.[66] MRK003 is a γ-secretase inhibitor that has

demonstrated in vitro anti-MM activity by blocking the Notch pathway.[67] There may be preclinical evidence that it also increases sensitivity to bortezomib,[68] and clinical evaluation is under consideration.

Hedgehog

The Hedgehog (Hh) pathway is necessary for cell growth and differentiation; its deregulation has been associated with several cancers, including MM.[69] NVP-LDE225 is a novel antagonist currently in development and has demonstrated an anti-MM activity in vitro by the downregulation of the Hh pathway.[70] Clinical studies in MM are also being considered and should soon be underway.

Kinesin Spindle Protein Inhibitors

Kinesin spindle protein (Ksp) inhibitor (ARRY-520) is a synthetic antimitotic agent that induces the death of actively dividing cells by targeting the Ksp, an essential component of mitosis. Ksp is a microtubule protein that is necessary in the formation of spindles.[71] ARRY-520 has shown promising activity not only as a single agent, but also in combination with bortezomib or lenalidomide in preclinical studies using MM cell lines and xenograft models.[72] These data also demonstrated the ability of ARRY-520 to downregulate mcl-1, a known driver in the development of dexamethasone resistance.[73] Based on phase I clinical studies in the RR setting,[74] a phase II study with ARRY-520 as a single agent and in combination with dexamethasone was carried out.[75] All patients had previously received an immunomodulator; 90% had received prior bortezomib, and 78% had prior autologous stem cell transplant. The most commonly reported treatment-emergent AEs were thrombocytopenia, anemia, neutropenia, and fatigue. The most frequent grade 3 or 4 AEs included neutropenia (62%) and thrombocytopenia (57%). Of the 32 patients in the single-agent arm, an MR or greater was observed in 6 patients (19%), 5 of which were PRs. Among patients who were bortezomib and lenalidomide refractory, a 15% ORR ($\geq$MR) was observed. Patients who received combination ARRY-520 and dexamethasone, the ORR was 28% (5 of 18), with 4 patients achieving a PR or greater.[75] There was no association between ARRY-520 and the development of peripheral neuropathy. Further evaluation of ARRY-520 in combination with other novel agents, such as bortezomib and carfilzomib, are underway.

Chromosome Region Maintenance 1

Chromosome region maintenance 1 (CMR1) is a nuclear export protein used to transfer proteins with leucine-rich nuclear sequences from the nucleus to the cytoplasm.[76] This shuttling system is very tightly regulated, given that its cargo includes tumor suppressor proteins, such as p53.[77,78]

The overexpression of CRM1 is responsible for the abnormal cellular localization of tumor suppressive proteins and has been implicated in the development of certain cancers.[79,80] Tai and colleagues[81] demonstrated that CRM1 is highly expressed in patients with MM, including those who are refractory to bortezomib. The overexpression of CRM1 was also correlated with lytic bone disease and a shorter survival. Irreversible selective inhibitors of nuclear export (SINE) targeting CRM1 (KPT-185, KPT-330) induced cell death in MM cells by the accumulation of CRM1 cargo tumor suppressor proteins, even in the presence of BMSCs or osteoclasts. In mice models with MM bone lesions, SINEs successfully inhibited bone lysis by impairing osteoclastogenesis and bone resorption by blocking the nuclear factor–κB pathway.[81] These results are convincing that CRM1 is an important therapeutic target and requires further investigation in human studies.

TARGETING THE BONE MARROW MICROENVIRONMENT

In MM, bone marrow microenvironmental factors play a crucial role in disease progression. Factors such as hypoxia, neoangiogenesis, and the critical interaction between plasma cells and bone marrow stroma are vitally important considerations when contemplating future drug targets in MM; these and other aspects of the tumor microenvironment, including the extramedullary milieu and cortical bone, constitute primary barriers to disease control.

Hypoxia

A hypoxic microenvironment has been associated with disease progression and drug resistance in MM.[82] TH-302 is a prodrug that is activated under tumor hypoxic conditions, a hallmark of MM where the bone marrow is often devoid of oxygen. TH-302 has demonstrated cytotoxicity against human cancer cell lines in vitro and was found to selectively target hypoxic MM cells in vivo, with promising early clinical activity seen in RR MM.[83]

Angiogenesis

Angiogenesis is governed by a balance between proangiogenic and antiangiogenic growth factors. Vascular endothelial growth factor (VEGF) is upregulated in MM. It is thought that active MM requires a vascular environment that contributes to clonal proliferation.[84] Studies looking at VEGF inhibitors have not been encouraging. Unfortunately, several phase II trials did not show any clinical responses in patients with RR MM.[85,86]

CXCR4

CXCR4 is a cell surface chemokine receptor expressed on the surface of normal and MM cells. The interaction between CXCR4 and its ligand stromal cell-derived factor 1 (SDF-1) plays a key role in adhesion and homing.[87] AMD3100 (plerixafor) is a CXCR4 inhibitor that blocks the interaction between MM cells and their bone marrow microenvironment.[88] Preliminary results from a phase I/II trial of plerixafor and bortezomib in RR MM demonstrated promising results.[89] Grade 3 toxicities included lymphopenia (40%), hypophosphatemia (20%), anemia (10%), hyponatremia (10%), and hypercalcemia (10%). Of the 10 evaluable patients, 1 (10%) achieved a VGPR, and 3 (30%) achieved PRs, with an ORR of 40% in this relapsed/refractory population.[89]

CELL CYCLE INHIBITORS

Aurora Kinase Inhibitors

The cell cycle is a very tightly organized process that involves the interaction of many regulatory proteins and enzymes. Aurora A kinases play a key role in the mitotic phase of the cell cycle by modulating chromosome configuration, spindle formation, and cytokinesis.[90–92] Inhibition of Aurora-A kinase gene expression in MM cells leads to apoptosis and cell death.[93,94] MLN8237 is the first orally available selective inhibitor of Aurora-A kinase. In preclinical studies, treatment of cultured MM cells with MLN8237 resulted in the inhibition of cell proliferation via apoptosis in addition to the upregulation of p53. When MLN8237 was combined with dexamethasone, doxorubicin, or bortezomib, anti-MM activity was amplified.[95] MLN8237 is currently under further investigation in phase I/II clinical trials in patients with RR MM, with encouraging tolerability and modest activity reported to date.

Cyclin-Dependent Kinase Inhibitors

Myeloma cells accumulate in the bone marrow because of impaired apoptosis.[96] Quiescent myeloma cells can become self-renewing by reentering the cell cycle, particularly during relapse. This deregulation of the cell cycle can be partially explained by the progression of MM cells through the G_1 phase by cyclin-dependent kinases (CDK).[97] Aberrant activation of Cdk4/Cdk6 is enhanced in advanced disease, regardless of previous treatment regimens or initial clinical presentation.[98] PD 0332991, an orally active inhibitor of recombinant Cdk4 and Cdk6, was shown to induce G_1 arrest in ex vivo myeloma cells and halted growth of tumor cells in human myeloma xenograft models.[99] Similarly, seliciclib (CYC202 or R-roscovitine) is a potent CDK inhibitor, which demonstrated compelling cytotoxicity against primary MM cells and cells resistant to conventional therapy. Seliciclib downregulated *Mcl-1* transcription and inhibited IL-6 transcription by tumor cells bound to BMSCs. The combination of seliciclib with bortezomib demonstrated synergism in vitro.[100] Early phase combination studies are now underway, and the results are anticipated with interest.

MONOCLONAL ANTIBODIES

Anti-CS1 (Elotuzumab)

CS1 is a cell surface glycoprotein and a member of the signaling lymphocyte-activating molecule-related receptor family (**Fig. 3**).[101] Using gene expression profiling, high CS1 expression was found in patients at all stages of MM, regardless of cytogenetics or previous lines of therapy.[102] There was little to no expression of CS1 in normal tissue,[102,103] which allows the opportunity for a potentially highly targeted therapy with a favorable therapeutic index.

Elotuzumab is a humanized monoclonal antibody (mAb) that targets CS1 and activates host natural killer (NK) cells to release perforin granules resulting in targeted myeloma cell death (see **Fig. 3**).[102,103] In preclinical studies, elotuzumab was successful in inducing antibody-dependent cell-mediated cytotoxicity (ADCC) in myeloma cells from patients known to be resistant to bortezomib[103,104] and also demonstrated inhibition of tumor growth in xenograft mouse models.[102,103] In other studies, the combination of elotuzumab with bortezomib or lenalidomide resulted in synergistically enhanced ADCC compared with any agent alone.[103,105]

Study 1703, a phase II trial, was conducted randomizing 73 patients with RR MM to either elotuzumab 10 mg/kg or 20 mg/kg IV once weekly in combination with lenalidomide at 25 mg (days 1–21) and low-dose oral dexamethasone.[106] At a median follow-up time of 20.8 months, the median PFS for elotuzumab 10 mg/kg arm was not reached, with a more recent estimate showing a median PFS of 33 months. Correlative studies confirmed equal saturation of the target at both doses on tumor obtained with serial bone marrow aspiration across treatment. Preliminary data established an ORR (PR or better) of 84% in all patients and 92% for patients treated with elotuzumab at a dose of 10 mg/kg IV. The median time to objective response was 1 month (range 0.7–19.2). Most common grade 3/4 treatment-emergent AEs were neutropenia (16%), thrombocytopenia (16%), and lymphopenia (16%). As commonly observed with other monoclonal antibodies, chills, pyrexia, flushing, and headache were the most common AEs. A premedication regimen with diphenhydramine, acetaminophen, and methylprednisolone reduced the incidence of infusion reactions subnstantially.[106] Based on these results, 10 mg/kg is now being taken forward in later phase studies.

Two phase III multicentered clinical trials have examined lenalidomide and low-dose oral dexamethasone with or without elotuzumab 10 mg/kg IV in patients with untreated MM (ELOQUENT 1) and in patients with RR MM (ELOQUENT 2). These trials

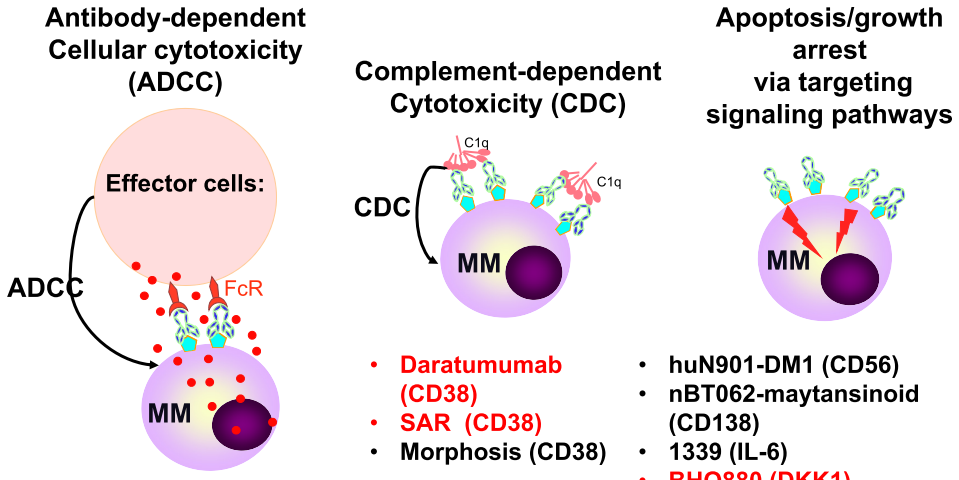

Fig. 3. Monoclonal antibody (MAb)-based therapeutic targeting of myeloma. SAR, SAR650984. (*Adapted from* Tai YT, Anderson KC. Antibody-based therapies in multiple myeloma. Bone Marrow Res 2011;2011:924058.)

assessed efficacy by measuring PFS, ORR, and overall survival. The studies are both completed and in the final stages of analysis, with results eagerly anticipated. Breakthrough status has been assigned to this agent because of these highly promising data, with regulatory approval hoped for within the next 12 months.

Anti-CD38

Daratumumab

CD38 is a 46-kDa single-chain, type II transmembrane glycoprotein with a short 20-aa N-terminal cytoplasmic tail and a 256-aa extracellular domain (see **Fig. 3**).[107] CD38 plays a role in receptor-mediated signaling events to regulate cell adhesion and also contributes to the intracellular mobilization of calcium.[108] CD38 is highly expressed on malignant plasma cells at all stages of MM.[109]

Daratumumab is a humanized monoclonal antibody targeting a unique epitope on the CD38 glycoprotein (see **Fig. 3**). It can effectively kill myeloma cells using ADCC, complement-dependent cytotoxicity (CDC), and apoptosis via cross-linking.[110] Preclinical studies demonstrated that daratumumab exhibited CDC and ADCC activity, even in the presence of BMSCs, which typically provide a protective microenvironment. The combination of lenalidomide and daratumumab demonstrated enhanced NK-mediated cytotoxicity in vitro using ADCC assays.[111]

In a phase I, first-in-human dose-escalation study, heavily pretreated patients with RR MM (median of 6 prior therapies; range 2–12) were treated with single-agent daratumumab over a period of 8 weeks. Ten cohorts were administered doses ranging from 0.005 mg/kg to 24.0 mg/kg. Of the 32 participants, 75% were refractory to both lenalidomide and bortezomib, 83% had previously undergone an autologous stem cell transplant, and 33% had undergone an allogeneic stem cell transplantation.[112–114] Preliminary efficacy data demonstrated a sizable reduction of bone marrow plasma cells by 80% to 100% in the cohorts receiving 4 mg/kg and onward. Overall, 42% of this heavily pretreated population of patients achieved at least a PR at doses 4 mg/kg or greater.[112]

Based on these very encouraging preliminary data, a phase I/II open-label multicenter study of daratumumab in combination with lenalidomide and oral dexamethasone is ongoing in the RR MM patient population. Daratumumab is being administered in dosages from 2 mg/kg to 16 mg/kg weekly for 8 weeks, twice a month for 16 weeks and once a month until disease progression, unmanageable toxicity or up to maximum 24 months. Preliminary data from 20 patients so far has shown a marked reduction in M protein, yielding a response rate of PR or better in 15 of the 20 patients (ORR 75%; CR = 3, VGPR = 6, PR = 6). Six AEs of grade 3 or more (5 events of neutropenia and 1 event of thrombocytopenia) were reported. Overall, daratumumab/lenalidomide/dexamethasone has also demonstrated a favorable safety profile with manageable toxicities, suggesting this combination has great promise for the future.[115] A broad range of studies in all phases are now underway as part of a comprehensive approval-finding strategy for daratumumab, and the agent was given breakthrough status in 2013.

SAR650984

SAR650984 (SAR) is a humanized IgG1 monoclonal antibody that also selectively targets the CD38 surface antigen on MM cells (see **Fig. 3**). SAR induces cell death by ADCC, CDC, and induction of apoptosis. SAR was investigated in a dose escalation phase I study in patients with selected CD38+ hematological malignancies, 27 of which had RR MM.[116] SAR was administered as a single-agent infusion every 2 weeks or weekly. After an initial accelerated dose-escalation schedule in phase I, all

subsequent dosages (0.3 mg/kg, 1 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, 20 mg/kg every 2 weeks and 10 mg/kg weekly) were administered following the classic 3 + 3 design based on DLT. DLTs were limited to grade 2 infusion reactions, which were mitigated with the introduction of a pretreatment regimen. The most common AEs were fatigue (46.9%), nausea (31.3%), pyrexia (28.1%), cough (25%), vomiting (21.9%), and hypercalcemia (18.8%), with headache, constipation, bone pain, chills, and diarrhea each occurring in 15.6% of patients. Responses included one PR at the 1 mg/kg (n = 3) and 5 mg/kg doses (n = 3). The 10 mg/kg dose demonstrated 3 PRs and 2 SDs among 6 patients with MM treated. The maximum tolerated dose was not reached with an every-other-week and an every-week schedule.[116] These data convincingly validate the targeting of CD38 in RR MM, and combination strategies are underway with particularly impressive response data already seen with lenalidomide and dexamethasone. Strategies combining SAR with pomalidomide and dexamethasone are now planned in advanced disease, targeting RR MM as an area of particular clinical need.

Anti–IL-6 (Siltuximab)

IL-6 is produced by stromal cells within the bone marrow and plays a crucial role in the proliferation and survival of MM cells.[117] IL-6 is implicated in chemotherapy resistance by its ability to protect against cell death.[118] Siltuximab is a chimeric monoclonal antibody targeting IL-6. Preclinical studies were encouraging with synergistic cytotoxic activity when siltuximab was combined with other agents, such as bortezomib,[118,119] and when considered as part of a rationale for targeting the tumor milieu.

A phase I dose-escalating study was conducted using single-agent siltuximab in relapsed/refractory patients. Although the drug was well tolerated, no responses were recorded.[120] A phase II trial evaluated siltuximab as a single agent and in combination with dexamethasone in RR MM.[121] As monotherapy, 62% of patients achieved SD at best; but when administered with dexamethasone, a PR rate of 19% and an MR rate 28% was seen, although the numbers were relatively small. Infections of any grade were seen in 57% of patients and grade 3 and 4 in 12% and 6% of patients, respectively.[121] A randomized phase II trial evaluated the addition of siltuximab to velcade, melphalan, prednisone (VMP) therapy in patients with untreated MM, but unfortunately no clinical benefit was seen.[122] Studies of siltuximab in other settings are being explored, including Castleman disease, with randomized studies showing a striking benefit in the latter, which suggests this agent may have a niche role when disease is highly IL-6 dependent.

NOVEL CYTOTOXICS

Melflufen

Melphalan-flufenamide (melflufen) is a prodrug that enhances the cytotoxic potential of melphalan by allowing a more rapid and superior incorporation of melphalan into the tumor cells resulting in intracellular hydrolysis and cell death.[123–125] Using in vitro and in vivo models, preclinical studies have demonstrated that melflufen has more potent antimyeloma activity than equimolar doses of melphalan and can induce apoptosis even in bortezomib and melphalan-resistant MM cells.[126] Melflufen exerts its anti-MM activity by the activation of caspases and through the induction of DNA damage. The combination of melflufen with other novel or conventional MM agents, such as bortezomib, lenalidomide, or dexamethasone, enhanced its cytotoxic effects.[126] These preclinical data provided the impetus for pursuing clinical trials evaluating the safety and efficacy of melflufen in the RR setting. An open-label phase I/IIa

study of melflufen in combination with dexamethasone in patients with relapsed or RR MM is currently ongoing. Early data have shown promising activity in this heavily pretreated population, with myelosuppression as one of the more commonly reported AEs but otherwise favorable tolerability to date.

SUMMARY

New, next-generation targeted treatment strategies are urgently required to improve outcomes in patients with MM. Monoclonal antibodies, cell signaling inhibitors, and selective therapies targeting the bone marrow microenvironment have demonstrated encouraging results with generally manageable toxicity in therapeutic trials of patients with RR disease, each critically informed by preclinical studies. A combination approach of these newer agents with immunomodulators and/or PIs as part of a treatment platform seems to consistently improve the efficacy of anti-MM regimens, even in heavily pretreated patients. Future studies continue to be required to better understand the complex mechanisms of drug resistance in MM. Incorporating molecular correlates to further personalize treatment and to, thus, better integrate these agents into clinical practice is a clear priority. There is a broad and very promising armamentarium, which also includes immune-based therapies (discussed elsewhere), now available against this hitherto incurable disease; the hope of durable long-term remission in an increasing proportion of our patients is, therefore, becoming a reality.

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