Published in final edited form as:

Leukemia. 2009 November ; 23(11): 1964–1979. doi:10.1038/leu.2009.173.

Proteasome Inhibitors in the Treatment of Multiple Myeloma

Jatin J. Shah[1] and **Robert Z. Orlowski**[1,2]

[1]The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma & Myeloma, Houston, TX

[2]The University of Texas M. D. Anderson Cancer Center, Department of Experimental Therapeutics, Division of Cancer Medicine, Houston, TX

Abstract

Targeting intracellular protein turnover by inhibiting the ubiquitin-proteasome pathway as a strategy for cancer therapy is a new addition to our chemotherapeutic armamentarium, and has seen its greatest successes against multiple myeloma. The first-in-class proteasome inhibitor bortezomib was initially approved for treatment of patients in the relapsed/refractory setting as a single agent, and was recently shown to induce even greater benefits as part of rationally-designed combinations that overcome chemoresistance. Modulation of proteasome function is also a rational approach to achieve chemosensitization to other anti-myeloma agents, and bortezomib has now been incorporated into the front-line setting. Bortezomib-based induction regimens are able to achieve higher overall response rates and response qualities than was the case with prior standards of care, and unlike these older approaches, maintain efficacy in patients with clinically- and molecularly-defined high-risk disease. Second-generation proteasome inhibitors with novel properties, such as NPI-0052 and carfilzomib, are entering the clinical arena, and showing evidence of anti-myeloma activity. In this spotlight review, we provide an overview of the current state of the art use of bortezomib and other proteasome inhibitors against multiple myeloma, and highlight areas for future study that will further optimize our ability to benefit patients with this disease.

Keywords

Bortezomib; carfilzomib; immunoproteasome; multiple myeloma; NF-κB; proteasome; NPI-0052

Introduction

Multiple myeloma is a neoplastic proliferation of plasma cells (1) which normally serve as engines for the large-scale synthesis of immunoglobulins. It is perhaps both ironic and fitting, therefore, that one of the most successful therapeutics against this disease disrupts

Address correspondence to: Dr. Robert Z. Orlowski, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma & Myeloma, 1515 Holcombe Blvd., Unit 429, Houston, TX 77030-4009, rorlowsk@mdanderson.org, Telephone 713-792-2860, Fax 713-563-5067.

Conflicts of Interest

J.J.S. is on the speaker's bureau for Millennium: The Takeda Oncology Company. R.Z.O. serves on advisory panels for Millennium: The Takeda Oncology Company, and Proteolix, Inc.

normal protein homeostasis by targeting the proteasome. As part of the ubiquitin-proteasome pathway, the proteasome is the final common effector of the vast majority of regulated intracellular proteolysis. Proteins destined for turnover are tagged with polyubiquitin chains by the ubiquitin conjugation system, which was elucidated by Nobel laureates Aaron Ciechanover, Avram Hershko, and Irwin Rose (2, 3). Target proteins are subject to turnover through the 20S and 26S proteasomes, which were characterized by Alfred Goldberg, Marian Orlowski, and Martin Rechsteiner (4–6). Each 20S particle, which also serves as the core of the 26S proteasome, contains up to five proteolytic activities which cleave proteins after acidic, basic, branched chain, hydrophobic, and small neutral amino acids, generating oligopeptides and, eventually, amino acids. Peptide inhibitors of the proteasome were initially synthesized to probe its proteolytic functions (7, 8). Interest in their potential as therapeutic agents was raised by later studies which determined that they induced apoptosis in both *in vitro* (9, 10) and *in vivo* (11, 12) tumor model systems.

The emergence of multiple myeloma as a rational target for proteasome inhibition was in part supported by pioneering studies showing the prominent role of the transcription factor nuclear factor kappa B (NF-κB) in the biology of this disease. As detailed in several excellent reviews (13, 14), NF-κB promotes myelomagenesis by inducing growth and angiogenesis factors such as interleukin (IL)-6 and vascular endothelial growth factor; by activating important cell cycle regulators such as c-Myc and Cyclin D1; by promoting an anti-apoptotic state through intermediates such as Bcl-2, and Bcl-x_L; and by enhancing myeloma cell adherence to the surrounding stroma such as through effects on fibronectin and vascular cell adhesion molecule-1. Proteasome inhibitors suppress NF-κB activity by stabilizing the inhibitory molecule IκB, which binds NF-κB and prevents its nuclear translocation, thereby down-regulating levels of its targets and producing a potent anti-myeloma effect (15). Notably, mutations that activate the canonical or non-canonical NF-κB pathway predict for a better response to bortezomib therapy (16, 17). In that the proteasome is involved in turnover of 80% or more of cellular proteins (18), proteasome inhibition also has a number of other effects. Many of these contribute to anti-tumor activity, such as by stabilizing pro-apoptotic p53 and Bax proteins, dissipating the mitochondrial transmembrane potential and inducing release of cytochrome c, activating c-Jun-N-terminal kinase (JNK), and stimulating endoplasmic reticulum (ER) stress. The latter may be especially important, in that some studies have suggested that the large basal level of ER stress associated with high levels of immunoglobulin production makes myeloma especially sensitive to proteasome inhibitors (19). Other effects of proteasome inhibitors appear to promote cellular survival, such as activating multiple heat shock protein (HSP) family members, inducing the stress response protein MKP-1, and promoting activity of the protein kinase B/Akt pathway (Table 1)(20). Fortunately, on balance, the net effect is typically a pro-apoptotic one, as evidenced by the findings of the first study of PS-341, now known as bortezomib, the first-in-class proteasome inhibitor to reach the clinic (21). All nine patients with plasma cell dyscrasias derived some benefit from therapy in this phase I trial, including one durable complete remission (CR), in part setting the stage for its further development.

Bortezomib in the relapsed/refractory setting

The anti-myeloma activity of bortezomib was initially confirmed in two multi-center phase II trials (22–25), the larger of which, led by Richardson and colleagues (22), administered bortezomib at the most commonly used dose and schedule, 1.3 mg/m^2 on days 1, 4, 8, and 11 of every 21-day cycle. Among 193 evaluable patients, of whom 91% had disease that was refractory to their previous therapy, partial response (PR) or better was seen in 27%, of whom 10% achieved a CR or near-CR (nCR). Many of these patients had never been in CR previously and, indeed, achieving CR was then a rare outcome in this setting. Also of note, the median time to progression (TTP) after bortezomib was 7 months, compared to 3 months on whatever had been the previous therapy. This also was a decidedly unusual and exciting outcome in myeloma, where response durability typically decreases with successive salvage regimens (26). Adverse events that reached at least moderate severity included thrombocytopenia, seen in 28% of patients, fatigue, seen in 12%, peripheral neuropathy, also in 12%, and neutropenia, seen in 11%. Bortezomib-mediated thrombocytopenia and peripheral neuropathy have since been extensively characterized, with the former being predictable and transient (27), and both being manageable and reversible (28–30). These findings led to the approval of bortezomib by the Food and Drug Administration for relapsed/refractory myeloma in patients with at least two prior lines of therapy.

Bortezomib was then studied in a randomized phase III trial in comparison with dexamethasone targeting relapsed myeloma (31). Toxicities were comparable to those seen in phase II, though the larger accrual and randomized design allowed for the identification of an increased, 13% risk of herpes zoster reactivation for bortezomib-treated patients (32). This finding has been confirmed in other trials (33), though its occurrence can be prevented with acyclovir (34). The initially reported response rate to bortezomib was 38%, which improved to 43% with continued therapy (35), and included CRs in 9%. These compared favorably to dexamethasone, where a PR or better was seen in only 18% of patients, with less than 1% CRs. Moreover, these improvements translated into superior durability, with a TTP of 6.22 months on bortezomib, and only 3.49 months with dexamethasone. As a result, median survival improved from 23.7 months for dexamethasone to 29.8 months for bortezomib (35). This was especially impressive in light of the fact that almost two-thirds of patients crossed over to bortezomib after a planned interim analysis showed it’s superiority. On the basis of these findings, bortezomib was approved for patients with relapsed and/or refractory myeloma who had received at least one prior therapy. Moreover, these data suggested that earlier bortezomib use was associated with a greater benefit, and pointed toward its adoption into front-line therapy.

Subsequent analyses of the data from these trials, as well as further clinical experience, revealed additional important findings that have guided bortezomib development, and informed our use of this drug. While the day 1, 4, 8, and 11 schedule remains the most commonly used, limited studies suggest that bortezomib given weekly, either alone at 1.6 mg/m^2 (36), or at 1.3 mg/m^2 with methylprednisolone (37), can be effective. Novel toxicities have been described in a minority of patients, including tumor lysis syndrome (38–40), and pulmonary complications (41, 42), the latter of which may be reduced with dexamethasone (43), as well as rare skin toxicity (44) and hepatitis (45). More common

drug-related toxicities, especially thrombocytopenia and peripheral neuropathy, appear to not be cumulative, allowing for extended dosing (46). Peripheral neuropathy may be due to the accumulation of aggresomes in dorsal root ganglia (47, 48), which may provide a mechanism-based approach to reduce its development. Other studies have suggested a possible contribution from increased tubulin polymerization and stabilization (49) and, in some cases, of immune-mediated mechanisms (50). Bortezomib was found to be safe and effective in patients with clinically-defined high-risk disease, including older patients (51), those with an elevated β_2-microglobulin (52), and those with renal failure (53–55). Indeed, bortezomib can be effective in contributing to a reversal of acute renal failure (56–58). Also, bortezomib can overcome molecularly-defined high-risk myeloma, especially disease characterized by deletion of chromosome 13 (59), and possibly by the 4;14 translocation (60, 61). Finally, a number of studies have shown the beneficial effects of bortezomib or bortezomib-based combinations on bone metabolism (62–66), including its ability to activate osteoblasts. Indeed, one analysis suggested that increases in bone alkaline phosphatase levels were a predictor of bortezomib response (67).

Bortezomib-based combination regimens

In addition to the efficacy of bortezomib as a single agent, proteasome inhibition is a rational approach to overcome chemoresistance, and achieve chemosensitization. Multiple mechanisms contribute to these endpoints, including suppression of chemotherapy-mediated NF-κB activation, inhibition of maturation of the P-glycoprotein multi-drug resistance pump, and induction of phosphorylation and cleavage of anti-apoptotic Bcl-2 into pro-apoptotic fragments, among others (Table 2)(20, 68, 69). A large number of bortezomib-based combinations have been tested pre-clinically, many of which have made their way into the clinic. Dexamethasone is one of the most common agents to be combined with bortezomib, based in part on pre-clinical studies showing that it increased anti-myeloma activity (15, 70). Several clinical trials have documented that dexamethasone addition improves the response rate and response quality in up to 34% of patients after a suboptimal benefit from single-agent bortezomib (22, 71, 72). Higher response rates have also been seen when this two-drug regimen is used initially (66, 73), as is the case for bortezomib with other corticosteroids (37), though randomized trials comparing these have not been performed.

One regimen for which randomized data are available is bortezomib with pegylated liposomal doxorubicin (Doxil®; PLD), which was designed based on studies showing anthracyclines suppressed induction of anti-apoptotic HSP and stress response proteins (74–76). A phase I trial showed this regimen was tolerable and active (77), with a greater response rate than that expected of bortezomib alone, and a better response durability, with a TTP of 9.3 months (78). These findings led to a pivotal randomized, phase III international study of bortezomib/PLD compared to bortezomib. This trial showed that addition of PLD significantly enhanced the response quality, with a 42% increase in the proportion of patients achieving a CR or very good partial remission (VGPR); the median duration of response, which increased from 7.0 months to 10.2; the median TTP, which rose from 6.5 to 9.3 months (79); and showed an early trend towards an improved overall survival (OS).

Bortezomib/PLD was also active in patients whose disease was refractory to other anti-myeloma agents (80), and was safe and effective in patients with renal compromise (81).

Other bortezomib-based combinations that have been studied for relapsed and/or refractory myeloma (Table 3) have included regimens incorporating alkylators, anthracyclines, HSP-90 inhibitors, histone deacetylase (HDAC) inhibitors, and monoclonal antibodies. Many of these have been rationally designed based on findings that proteasome inhibition suppresses DNA damage repair pathways (70), supporting combinations with DNA damaging agents; activates the anti-apoptotic HSP and stress responses (82, 83), as well as the pro-survival protein kinase B/Akt (84, 85), supporting regimens with HSP inhibitors such as tanespimycin and the anti-IL-6 monoclonal antibody CNTO 328 (83); and induces aggresome formation as an alternative means to remove misfolded proteins (86, 87), supporting the use of HDAC inhibitors which block this pathway. Studies of these regimens have generally found that bortezomib can be delivered at, or very near its standard dose and schedule, even with the addition of other drugs, typically with little, if any, overlapping toxicities. Several of these have suggested that bortezomib-based combinations are associated with lower rates of neuropathy (79), in some cases possibly due to decreased aggresome formation (88) in dorsal root ganglia. In addition, these studies have generally shown encouraging signs of activity, including response rates approaching 100% in some cases (89, 90), and in others the ability to induce responses in patients whose disease was previously bortezomib-refractory (91–95). Pivotal trials that could lead to regulatory approvals are underway or planned with several of these, including bortezomib with CNTO 328, perifosine, tanespimycin, and vorinostat.

Bortezomib for previously chemotherapy-naïve multiple myeloma

Validation of the activity of bortezomib against myeloma quickly prompted investigators to incorporate it into the front-line setting to improve the efficacy of induction therapy. The first such study, led by Jagannath and colleagues, evaluated bortezomib alone at the relapsed/refractory dose and schedule, to which dexamethasone was added at 40 mg on each day of bortezomib therapy, and the day after, if less than a PR was achieved after two cycles (96). Common adverse events of at least grade 2 severity included sensory neuropathy, seen in 31% of patients, constipation, seen in 28%, myalgias, also in 28%, and fatigue, seen in 25%. Bortezomib alone produced an overall response rate of 40% in 32 patients, including 12% who achieved a CR or nCR, and after addition of dexamethasone, the response rate improved to 88%, including 25% with CR/nCR. Activity of the bortezomib/dexamethasone regimen was confirmed by a later phase II study, which found a response rate of 66% using lower corticosteroid doses (97). A large number of other bortezomib-based combinations have since been evaluated (Table 4), and response rates of 90% or better, and CR rates of 30% or more prior to transplantation have been achieved. Notable examples include regimens that have added anthracyclines, including bortezomib with infusional doxorubicin and dexamethasone (PAD; (98, 99)), or bortezomib with PLD/dexamethasone (100, 101); combinations that have added immunomodulatory drugs, including bortezomib with thalidomide and dexamethasone (TD; (102)) or lenalidomide and dexamethasone (103); and groupings with cytotoxic agents, such as bortezomib with cyclophosphamide (104, 105), and bortezomib with dexamethasone, continuous-infusion cisplatin, doxorubicin,

cyclophosphamide, etoposide, and daily thalidomide (DT-PACE; (106)), or with DT-PACE in the setting of Total Therapy III (107).

Phase III trials of these regimens are underway, or in some cases have been completed, comparing modern induction therapy with older standards of care (Table 4). Harousseau and colleagues have reported on their study randomizing patients to bortezomib/dexamethasone or infusional vincristine and doxorubicin with oral dexamethasone (VAD; (108)). Patients receiving bortezomib/dexamethasone had a better overall response rate and response quality, with 39% achieving at least a VGPR, compared to only 16% after VAD. This edge was maintained after transplantation, with 61% reaching a VGPR or better if bortezomib/dexamethasone was used, versus only 44% after VAD. Moreover, since a second transplant was added to patients who did not reach a VGPR after their first transplant, more patients induced with VAD required this additional therapy. Despite this, the early follow-up data indicate a superior progression-free survival (PFS) and fewer deaths among the group induced with bortezomib/dexamethasone. Important findings have also been reported by Cavo and colleagues, who have compared bortezomib with thalidomide and dexamethasone (VTD) to TD (109). Induction therapy with VTD provided a 62% VGPR or better rate compared to only 29% for TD, and after transplantation this benefit was maintained, with 76% having VGPR or better if they started with VTD, compared to only 58% of those who had received TD. Notably, both studies found no impact on stem cell mobilization, collection, and later engraftment by the addition of bortezomib, as has been the case for other pre-transplant trials. Also, both found that the bortezomib-based therapy remained superior in patients with high-risk disease, including patients with an elevated β2-microglobulin (108), deletion of chromosome 13 (108, 109), the 4;14 translocation (109), or deletion of p53 (109).

For transplant-ineligible patients, bortezomib-based therapies that have been studied include bortezomib with MP (110, 111), with thalidomide and prednisone (112), and with MP and thalidomide (113), among others (Table 4). Mateos and colleagues reported the results of a phase I/II trial of bortezomib with melphalan and prednisone (VMP), whose principal toxicities included thrombocytopenia, seen in 51% of patients, neutropenia in 43%, peripheral neuropathy in 17%, and diarrhea in 16% of this older population (110). A response rate of 89% was noted, including 32% with immunofixation-negative CR, half of whom were also in remission by immunophenotyping. Longer-term follow-up showed a median TTP of 27.2 months, which was not influenced by poor-risk features such as an elevated β2-microglobulin or adverse cytogenetics (111). These findings provided the rationale for an international, randomized phase III study comparing VMP with MP, which revealed the three-drug regimen induced a 71% overall response rate and CRs in 30%, compared to only 35% and 4%, respectively, for MP (114). Time to progression was the primary study endpoint, and proved to be 24.0 months for VMP, compared with 16.6 months for MP, while the median response duration was prolonged from 13.1 months for MP, to 19.9 for VMP. Importantly, though the median OS had not been reached on either arm, a significant trend was emerging favoring VMP, with a hazard ratio of 0.64 after a median follow-up of 25.9 months.

Bortezomib in other myeloma-related settings

A number of investigators have begun to use bortezomib or a bortezomib-based combination as a consolidation or maintenance strategy. One such study found that bortezomib/dexamethasone after a response to salvage therapy for relapsed and/or refractory disease converted six PRs into either VGPRs or CRs, and decreased M-protein values in 11/40 patients (115). Median TTP was 23 months with a PFS of 69% at 1-year, and an OS of 63% at 1-year, though four patients died of infectious complications. Another trial focused on high risk patients who received both induction with bortezomib as a single-agent, providing a 48% PR rate, and then followed this with maintenance bortezomib, which upgraded one patient to a CR (116). Among fifteen patients who received maintenance, the median PFS was 19.8 months though, notably, among seven who progressed on maintenance and were reinduced with bortezomib, none responded.

In the transplant setting, several investigators have evaluated incorporating bortezomib into the conditioning regimen. At the University of Arkansas, bortezomib was dosed at 1.0–1.3 mg/m^2 on days −4 and −1 prior to melphalan, which was administered at up to 250 mg/m^2 in fractionated doses (117). This approach was safe, with non-hematologic toxicities of at least grade 3 including mucositis, diarrhea, febrile neutropenia, pneumonia/sepsis, and fatigue. Of 27 evaluable patients, which consisted of a high-risk population with poor risk cytogenetic features and up to 75% having had prior transplantation, PR was obtained in 9 (39%), including 6 (26%) who achieved a CR. Another study focusing on high-risk patients treated those with primary refractory myeloma and plasma cell leukemias with two cycles of bortezomib, then high dose melphalan and bortezomib conditioning, and finally tandem transplantation (118). No dose-limiting toxicities (DLTs) were seen, and an overall response rate of 90% was noted, including 53% in VGPR or better. In lower risk patients with relapsed myeloma, bortezomib provided an even higher VGPR or better rate of 87% in 15 patients (119). More intense dosing of bortezomib was studied by L'Intergroupe Francophone du Myelome, who added 1.0 mg/m^2 of bortezomib on days −6, −3, +1 and +4 to high-dose melphalan on day −2 (120). Among 57 patients, this resulted in mucositis in 39%, erythroderma in 30%, headache in 20%, hallucinations in 9%, and grade 2 peripheral neuropathy in 3 cases, as well as five serious adverse events, including pulmonary embolism in one patient, seizure in one, acute cholecystitis in one, and two cases of pneumonia. Twenty patients (36%) achieved a CR and another 17 (30%) a VGPR, and further studies of this regimen are planned. Importantly, the impact of bortezomib scheduling may require further study, since one trial evaluated giving bortezomib 24 hours before or after high-dose melphalan (121). While there were no differences in toxicities or engraftment, bortezomib after melphalan produced higher levels of apoptotic indexes in the marrow, suggesting the possibility of a greater myeloma cell kill.

Greater experience has been garnered using bortezomib in maintenance or consolidation after prior transplantation. One common finding is that bortezomib produced a higher rate of reactivation of herpes zoster (122–126) than in the relapsed and/or refractory setting, indicating a definite need for prophylaxis. Several single-arm studies have shown that bortezomib produced high overall response rates (123, 125, 127), an improvement in response quality (124, 126), and an increased PFS (123, 125, 127). In one notable trial, 62

patients received induction therapy with VAD, followed by VTD, and then bortezomib maintenance after autologous transplantation. Complete remission or nCR was seen in 68% of patients, who had a 98% 1-year OS (127). Another interesting approach applied VTD as a consolidation in 40 patients who were in CR or VGPR after transplantation, and were thalidomide- and bortezomib-naïve (128). Six converted into a molecular remission based on studies of an immunoglobulin heavy chain gene rearrangement. Notably, none showed evidence of clinical relapse with a median follow-up of 26 months, while eight relapses were seen in the group that did not achieve molecular remission with a median of 12 months.

Control of graft-versus-host disease (GVHD) may be another potential application for bortezomib. This is supported by pre-clinical studies showing bortezomib's ability to inhibit *in vitro* mixed lymphocyte responses and promote apoptosis of alloreactive T cells, resulting in protection from acute GVHD without reducing graft-versus-leukemia effects (129). Interestingly, this depended on the timing of bortezomib administration, and was seen if it was given immediately after transplantation (129), whereas delayed administration exacerbated GVHD (130). The latter is supported by clinical data from one report showing a mild aggravation of existing acute or chronic GVHD in several patients, and appearance of *de novo* GVHD in one, when bortezomib was used after allogeneic transplantation (126). Other studies, however, have reported bortezomib could be safely given after prior allografting without exacerbating GVHD, and showed the ability to improve survival in responding patients (131), and to even control chronic GVHD (132).

Retreatment with, and resistance to bortezomib

The incorporation of bortezomib into the up-front setting will provide significant benefits to patients requiring induction chemotherapy. However, most of the data in the relapsed and/or refractory setting were obtained in bortezomib-naïve patients, and these findings may therefore be less applicable to cohorts who have been previously proteasome inhibitor-exposed. Fortunately, there are some data on retreatment in patients who have been previously bortezomib-exposed. One retrospective study of 22 patients who had achieved a 68% response rate with bortezomib alone or with dexamethasone on the phase II or III trials targeting relapsed and/or refractory disease, found that retreatment produced a 50% response rate (133). A second, larger study of 82 patients who had achieved an initial response rate of 59%, found that retreatment induced a 22% overall response rate (134). Notably, patients who had achieved at least a VGPR previously were more likely to respond on rechallenge, and had a 44% response rate. An interesting approach may be to start with bortezomib alone, and then to add other drugs if the response is less than brisk, such as dexamethasone, or MP, which resulted in a 73% response rate in one study (135).

While retreatment, especially with combination regimens, appears to be an attractive option, the decreased responses rate seen on rechallenge indicates the emergence of resistance. Elucidation of these mechanisms is an important goal to identify approaches to subvert them and resensitize myeloma to bortezomib, and to possibly find approaches to prevent them from arising altogether. Early reports indicate that increased and/or altered proteasome subunit expression may play a role (136, 137). This appears to be especially the case for the β5 proteasome subunit, which contains the chymotrypsin-like activity and is the major target

for bortezomib. Its overexpression (137–139) and mutation (139, 140) has induced bortezomib resistance in several *in vitro*-derived models, and studies to determine if similar mechanisms may be relevant in the clinical arena are underway. These findings suggest that proteasome inhibitors that bind irreversibly, and/or to a greater number of proteasome subunits with a more profound impact on proteolysis, could be of interest. Some support has been obtained for this in studies of NPI-0052 (141) and carfilzomib (142), both of which are irreversible inhibitors that have overcome bortezomib resistance in pre-clinical models. Other investigators have reported the presence in primary cells of proteasome inhibitor-resistant pathways of NF-κB activation (143, 144), suggesting that combination regimens with other strategies to suppress NF-κB, such as with IκB kinase inhibitors (145), may prove fruitful. Finally, knockdown of multi-drug resistance mechanisms, including the P-glycoprotein (146), and mitochondria-mediated anti-apoptotic pathways (147), have also been noted to overcome bortezomib resistance pathways, providing many attractive avenues for further research.

Interactions between bortezomib and dietary supplements

Most proteasome inhibitors are based on peptides, which serve as active site analogues that bind the proteasome subunits, and also contain a chemical entity, or “warhead,” which binds to the active site threonine. Julian Adams presciently selected boronic acid as the bortezomib warhead due to its ability to greatly enhance potency and specificity in comparison with older agents such as peptidyl-aldehydes (148, 149). However, boronates also interact with compounds that contain 1,2- or 1,3-diols to form cyclic moieties (150) in which the boronic acid is no longer free to bind the proteasome. Consistent with this possibility, vitamin C, a 1,2-diol, has been described to bind and inactivate bortezomib, thereby reducing its pre-clinical anti-cancer activity (151). Similarly, epigallocatechin gallate (EGCG), a polyphenolic green tea component, has comparable capabilities (152). These findings have led to recommendations that patients receiving bortezomib should limit their intake of vitamin C, EGCG, and related anti-oxidants (153). This advice is likely to be especially applicable for patients who take pharmacologic doses of such supplements (154, 155), highlighting the importance for health-care providers to make a careful and regular inventory of the agents being used by their patients. Finally, any maneuvers that increase reactive oxygen species (ROS) levels could contribute to the oxidative deboronation of bortezomib mediated by cytochrome P450 (156), and therefore potentially reduce anti-myeloma activity, though this could be negated by the pro-apoptotic activities of ROS (157). Notably, proteasome inhibitors with different chemistries, such as epoxyketones, would be expected to not be affected in this fashion (153).

Novel proteasome inhibitors

With validation of the proteasome as a target for myeloma therapy, interest was enhanced in the development of inhibitors that could have novel, attractive features. A number of such agents have been validated in the pre-clinical setting, most notably NPI-0052 (141, 158) and carfilzomib (142, 159). NPI-0052, also known as salinosporamide A, is related to one of the first proteasome inhibitors identified in nature, lactacystin (160). This agent was found to bind irreversibly to the subunits responsible for the chymotryptic and tryptic proteasome

activities, thereby inducing apoptosis through activation of caspase-8 with enhanced potency compared to bortezomib (141). Perhaps due to these properties, NPI-0052 was able to overcome bortezomib resistance and, interestingly, acted synergistically with bortezomib (158). Preliminary findings of a phase I study of this agent targeting myeloma patients have shown it to be well tolerated using weekly intravenous injection for three consecutive weeks out of four (161). Toxicities were comparable to those of bortezomib, but notable for an apparent lack of neurotoxicity, though one patient did develop reversible renal insufficiency. Stable disease was seen as the best response in several patients with previously progressing myeloma, and further studies are underway.

Carfilzomib is a peptide epoxyketone derived from epoxomicin (162) which, like NPI-0052, binds irreversibly to the chymotrypsin-like activity, and may be more proteasome-targeted than bortezomib (163). Preclinical studies showed that carfilzomib was more potent in its ability to induce caspase-8 and caspase-9 than bortezomib, and could overcome bortezomib resistance in cell line and primary plasma cell models (142). Moreover, carfilzomib could be dosed on consecutive days in pre-clinical *in vivo* models without enhanced toxicity, and proteasome inhibition in excess of 80% could be achieved (159), distinguishing this agent from bortezomib. Starting with the hypothesis that carfilzomib could therefore achieve more prolonged, and perhaps more profound proteasome inhibition, and that this could result in enhanced efficacy, two phase I studies targeting B-cell-derived malignancies have now been completed. One of these used a regimen of daily dosing for five consecutive days followed by nine days off (164), while the other dosed carfilzomib daily for two days of three consecutive weeks, followed by twelve days off (165). The maximum tolerated dose (MTD) for the five consecutive day regimen proved to be 15 mg/m^2, with DLTs consisting of febrile neutropenia and grade 4 thrombocytopenia, while other grade 3–4 events were primarily hematologic. Stable disease or better was achieved by six myeloma patients, including one who experienced a PR after having previously bortezomib-refractory disease (164). When carfilzomib was dosed on days 1, 2, 8, 9, 15, and 16 every 28-days, the MTD was 20 mg/m^2, with a hypoxic event noted as the DLT above this level. In addition, three of five patients dosed at 27 mg/m^2 experienced an increase in creatinine of up to grade 2 severity that was typically associated with a rapid decline in M-protein, but without evidence of tumor lysis syndrome, and which did not recur after repeat dosing. Four myeloma patients achieved at least a PR (165), including some whose disease had been previously refractory to bortezomib-based combinations.

Phase II studies of carfilzomib are currently underway using the latter dosing schedule targeting patients with relapsed and refractory (166) or refractory myeloma (167). In the former group, common adverse events included fatigue, nausea, upper respiratory infection, and diarrhea, while worsening of hematologic parameters was predominantly of grade 1 or 2 severity. An increased creatinine was seen in 15/46 patients, including four with acute renal failure, which in some patients may have been associated with tumor lysis, but led to dose discontinuation in only three patients. Among 39 evaluable patients, all of whom had received prior bortezomib, ten (26%) achieved at least a minor response or better, including five with PRs, and sixteen additional patients had stable disease (166). In relapsed patients, non-hematologic and hematologic toxicities were similar, and an increased creatinine was

seen in 5 patients (16%), with two cases of possible tumor lysis that led to one patient discontinuing therapy and one death (167). Of note, after modification of both trials to incorporate tumor lysis prophylaxis, renal events have become rare. Also, despite a high rate of baseline neuropathy in both patient populations, reports of worsening neuropathic symptoms were uncommon. Carfilzomib was active in patients with bortezomib-naïve disease, inducing a PR or better in at least 54% of thirteen evaluable patients, including one CR, and also in bortezomib-exposed patients, among whom 3/16 (19%) achieved a PR. Time to progression was 169 days in the latter group, and the median had not yet been reached for the former.

Conclusions and future directions

Inhibition of the proteasome as a strategy against multiple myeloma has revolutionized the care of patients afflicted with this malignancy, and contributed significantly to the increasing overall survival that is now seen in comparison with historical controls (168, 169). Use of bortezomib, the first-in-class proteasome inhibitor, as a single agent, and of bortezomib with dexamethasone, is a standard of care and widely accepted strategy, respectively, against relapsed and/or refractory myeloma. The regimen of bortezomib with PLD has been validated in a phase III trial as being superior to bortezomib alone, and other pivotal trials of bortezomib-based combinations are underway (Table 6). Thus, it is very likely that, in the near future, we will have several doublet regimens to select from in the relapsed and/or refractory setting. Studies will then be needed comparing these combinations to determine their relative risks and benefits, and to evaluate the possibility that baseline molecular aspects of each patient's disease would help to predict which could be most effective. Bortezomib has now been moved into the up-front setting, and received regulatory approval as an induction therapy in combination with MP for transplant-ineligible patients. Moreover, completed and ongoing studies (Table 6) in transplant-eligible populations will probably lead to additional approvals by proving bortezomib-based therapies to be superior to previous standards such as VAD and TD. Attractive features of bortezomib in all these settings include its efficacy and safety in clinically- and molecularly-defined high risk disease, its beneficial effects on bone metabolism, and especially its ability to augment the efficacy of other anti-myeloma agents in an additive to synergistic manner with an acceptable toxicity profile.

Despite these advances, much remains to be learned about the role of proteasome inhibitors in multiple myeloma. Bortezomib has been shown to be safe for consolidation or maintenance after prior standard- or high-dose therapy, for incorporation into pre-transplant conditioning, and for treatment of GVHD. However, appropriately powered, randomized studies with long-term followup are needed to validate the early parameters of efficacy that have been obtained. Of greatest concern is the possibility that, with the use of bortezomib-based induction therapy, patients at relapse will have disease that is less responsive to rechallenge with bortezomib-containing combinations. Early studies of retreatment strategies after prior therapy of relapsed/refractory disease (Table 5) are somewhat reassuring, in that they show that there is definitely a subgroup of patients who can benefit from the reuse of bortezomib. Moreover, it is possible that in a first-relapse setting, myeloma will be more responsive to bortezomib-based therapy after induction with

bortezomib than is the case in the relapsed/refractory setting, where patients have received multiple prior lines of therapy. Some support for this is provided by initial data of the results of bortezomib use in the salvage setting after prior induction with VMP (170), showing that the overall and CR rates are comparable to those seen in the bortezomib-naïve relapsed/refractory setting. Again, however, appropriately powered, randomized studies with long-term follow-up will be needed to validate these approaches. Moreover, the fact that response rates at rechallenge in patients with previously bortezomib-sensitive disease are typically lower than in the previous line, indicates the emergence of resistance to bortezomib in at least a subpopulation. A greater understanding of the mechanisms behind this phenomenon at the clinical level is urgently needed to help identify strategies that may be successful in overcoming such resistance. Since multiple mechanisms are likely to contribute to this phenotype, by studying which pathways are activated in their particular myeloma, it may be possible to individualize therapy for such patients. In that bortezomib in combination with Akt (91), HDAC (94, 171), or HSP-90 (93) inhibitors has in some cases been effective in patients with previously bortezomib-refractory disease, it is possible that we already have some of the necessary agents at our disposal to resensitize myeloma to bortezomib.

Another successful approach in this setting may be to switch to a different proteasome inhibitor, in analogy with data on the use of immunomodulatory agents against myeloma (172–174), which show that, for example, lenalidomide can be effective despite prior thalidomide use (175). Encouraging pre-clinical data have been obtained in this regard with NPI-0052 and carfilzomib, which utilize different chemistries than bortezomib, bind the proteasome irreversibly, and may inactivate more proteasome activities *in vivo*. Both of these have entered clinical trials and are showing good tolerability, as well as early suggestions that they may induce a lower rate of peripheral neuropathy. Carfilzomib in particular, which has advanced further in development, is showing encouraging activity as a single agent in both bortezomib-naïve and bortezomib-exposed populations. However, the former have generally shown a better response rate, supporting the possibility that there may be at least some cross-resistance between all proteasome inhibitors. Moreover, as is the case for bortezomib, combination regimens based on carfilzomib and NPI-0052 will probably prove most effective against myeloma. It is likely that additional proteasome inhibitors with novel properties may yet find applicability as part of our chemotherapeutic armamentarium against multiple myeloma. Several inhibitors that will be dosed using the oral route are now entering the clinical arena, including both boronic acid (176, 177) and epoxyketone-based agents (178). Also, immunoproteasome-specific inhibitors (179) that would be likely to have less associated non-hematologic toxicity due to the relatively restricted expression of the immunoproteasome subunits to hematopoietic tissues, may represent another attractive class of agents. In conclusion, it is likely that proteasome inhibitors will form an increasingly important part of our attack against multiple myeloma, and will contribute prominently to our ultimate endpoint of curing patients of this malignancy.

Acknowledgments

R.Z.O., a Leukemia & Lymphoma Society Foundation Scholar in Clinical Research, would also like to acknowledge support from the Leukemia & Lymphoma Society (6096-07), and the National Cancer Institute (RO1 CA102278).

Abbreviations

CR	complete remissions
D-PACE	dexamethasone, continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide
DT-PACE	dexamethasone, continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide with daily thalidomide
DLTs	dose-limiting toxicities
EBMT	European Group for Blood & Marrow Transplantation
ECOG	Eastern Cooperative Oncology Group
EGCG	epigallocatechin gallate
GVHD	graft-versus-host disease
HDAC	histone deacetylase
HSP	heat shock protein
IL	interleukin
JNK	c-Jun-N-terminal kinase
MTD	maximum tolerated dose
nCR	near-complete remission
NF-κB	nuclear factor kappa B
NMSG	Nordic Myeloma Study Group
NR	not reported
ORR	overall response rate
OS	overall survival
PAD	bortezomib with infusional doxorubicin and dexamethasone
PETHEMA	Programa para el Estudio de la Terapéutica en Hemopatía Maligna
PFS	progression-free survival
PLD	pegylated liposomal doxorubicin
PR	partial remissions
ROS	reactive oxygen species
SWOG	Southwest Oncology Group
TD	thalidomide and dexamethasone
TTP	time to progression
VAD	infusional vincristine and doxorubicin with dexamethasone
VBAD	vincristine with BCNU, Adriamycin, and dexamethasone

VBMCP	vincristine with BCNU, melphalan, cyclophosphamide, and prednisone
VGPR	very good partial remission
VMP	bortezomib with melphalan and prednisone
VTD	bortezomib with thalidomide and dexamethasone

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Table 1

Overview of Some of the Molecular Effects of Proteasome Inhibitors That Contribute to Their Anti-myeloma Activity

Target	Mechanism	Consequence	References
α4 integrin	Downregulate expression of VLA-4	Overcome adhesion-mediated drug resistance	(180)
BH3 proteins	Stabilize BIK, NOXA and BIM	Contribute to activation of Bax and Bak	(181–183)
Calcium	Dysrupt mitochondrial calcium uniporter	Dysregulate intracellular calcium storage; induce caspase activation	(184)
Caveolin 1	Inhibit VEGF-triggered caveolin phosphorylation; decrease caveolin expression	Reduce myeloma cell migration and survival	(185)
Cdkis	Stabilize Cdkis such as p21 and p27	Arrest the cell cycle	(15)
HIF-1α	Stimulate FIH	Inhibit tumor angiogenesis and tumor adaptation to hypoxia	(186)
HLA	Down-regulate surface expression of class I molecules	Enhance natural killer cell- mediated lysis of myeloma cells	(187)
HSP-90	Induce HSP-90 expression and cell surface exposure	Enhance dendritic cell-mediated induction of immunity	(188)
IL-6	Reduce stromal cell production of IL-6 through NF-κB Also down-regulate gp130 through caspase-mediated process	Suppress IL-6-mediated growth and survival signals	(189, 190)
IGF-1	Down-regulate IGF-1 and IGF-1R expression	Suppress IGF-1-mediated growth and survival signals	(82)
JNK	Activate JNK	Upregulate Fas and activate caspase-8 and caspase-3	(82)
Mcl-1	Induce Mcl-1 cleavage	Reduce anti-apoptotic Mcl-1; induce, pro-apoptotic Mcl-1 fragments	(183, 191)
MKP-1	Induce MKP-1 expression	Inhibit p44/42 MAPK-mediated growth and survival signals	(74, 76)
NF-κB	Stabilize IκB	Multiple mechanisms; please see text for more details	(70, 192)
p53	Cause accumulation and phosphorylation of p53	Induce downstream targets such as p21, NOXA, and Bax	(193)
ROS	Induce reactive oxygen species production	Promote mitochondrial injury with release of pro-apoptotic factors	(157)
UPR	Induce pro-apoptotic UPR genes; suppress anti-apoptotic UPR responses	Activate caspase-mediated apoptosis	(86, 194, 195)
VEGF	Suppress stromal cell production of VEGF	Reduce myeloma cell migration and marrow angiogenesis	(196)

Abbreviations: Bak, Bcl-2 homologous antagonist/killer; Bax, Bcl-2–associated X protein; BH3, Bcl-2 homology domain 3; BIK, Bcl-2 interacting killer; Cdkis, cyclin-dependent kinase inhibitors; Fas, tumor necrosis factor receptor superfamily, member 6; FIH, factor inhibiting HIF-1α; HLA, human leukocyte antigen; HIF, hypoxia-inducible factor; HSP, heat shock protein; IGF, insulin-like growth factor; IGF-1R, IGF-1 receptor; IκB, inhibitor of NF-κB; IL, interleukin; JNK, c-Jun-N-terminal kinase; MAPK, mitogen-activated protein kinase; Mcl, myeloid cell leukemia; MKP, mitogen-activated protein kinase phosphatase; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; UPR, unfolded protein response; VEGF, vascular endothelial growth factor; VLA, very late antigen

Table 2

Overview of Additional Mechanisms of Action of Proteasome Inhibitors That Add to the Anti-myeloma Activity of Other Chemotherapeutics

Target	Mechanism	Consequence	References
Bcl-2	Induce Bcl-2 phosphorylation and cleavage	Sensitize to multiple cytotoxic agents	(157, 197)
DNA-PK	Suppress expression, and induce cleavage, of DNA PK and other DNA damage repair enzymes	Sensitize to DNA damaging agents such as alkylators and anthracyclines	(70, 193)
NF-κB	Stabilize IκB	Sensitize to multiple cytotoxic agents	(70, 192)
PgP	Inhibit normal maturation of precursor forms of PgP	Reduce multi-drug resistance to chemotherapeutics subject to PgP	(146, 198, 199)
Survivin	Reduce survivin levels in combination with cytotoxics	Sensitize to DNA damaging drugs	(200)
Topo-I	Prevent tumor-induced degradation of Topo-I	Sensitize to agents that inhibit topoisomerase I	(201)
Topo-IIα	Stabilize Topo-IIα	Sensitize to agents that inhibit topoisomerase IIα	(202)

Abbreviations: Bcl-2, B-cell CLL/lymphoma-2; DNA-PK, DNA-dependent protein kinase; IκB, inhibitor of NF-κB; NF-κB, nuclear factor kappa B; PgP, P-glycoprotein; Topo, topoisomerase

Table 3

Bortezomib-based Combination Regimens in the Treatment of Relapsed and/or Refractory Multiple Myeloma*

Regimen	Study Phase	Response Rates‡,§	Reference
Two-drug regimens			
Bortezomib + pegylated liposomal doxorubicin	I	73% (n=22)	(77)
Bortezomib + melphalan	I/II	47% (n=34)	(203, 204)
Bortezomib + samarium lexidronam	I	13% (n=24)	(205)
Bortezomib + perifosine	I/II	16% (n=57)	(91)
Bortezomib + tanespimycin (cremophor formulation)	I/II	46%‡ (n=40)	(92)
Bortezomib + tanespimycin (suspension formulation)	II	NR^ (n=63)	(93)
Bortezomib + vorinostat	I	26% (n=34)	(94)
Bortezomib + panobinostat	I	36%† (n=14)	(171)
Bortezomib + CNTO 328	II	57% (n=21)	(206)
Three-drug regimens			
Bortezomib + thalidomide, dexamethasone	II	53% (n=18)	(207)
Bortezomib + thalidomide, dexamethasone	I/II	63% (n=85)	(208)
Bortezomib + ascorbic acid, arsenic trioxide	I	9% (n=22)	(209)
Bortezomib + cyclophosphamide, dexamethasone	II	66% (n=50)	(210)
Bortezomib + cyclophosphamide, dexamethasone	II	95%‡ (n=37)	(90)
Bortezomib + doxorubicin, dexamethasone	II	67% (n=64)	(211)
Bortezomib + lenalidomide, dexamethasone	II	67% (n=63)	(95)
Bortezomib + vorinostat, dexamethasone	I	43% (n=21)	(94)
Four-drug regimens			
Bortezomib + melphalan, prednisone, thalidomide	I/II	67% (n=30)	(212)
Bortezomib + melphalan, dexamethasone, thalidomide	II	66% (n=62)	(65)
Bortezomib + liposomal doxorubicin, thalidomide, dexamethasone	II	81% (n=42)	(89)
Other regimens			
Bortezomib + intermediate-dose melphalan (100 mg/m^2), thalidomide, dexamethasone, and stem cell support	II	65% (n=26)	(213)

*Due to space limitations, the authors have chosen to emphasize those studies that have appeared in peer-reviewed format, and we apologize in advance to our many colleagues who have reported excellent work in abstract form that has not yet been published which was omitted.

‡Response rates shown are overall responses, including complete + partial responses, unless otherwise indicated by‡, in which case the response rate includes minor responses.

§The study evaluable population is indicated in parentheses.

^NR, not reported

†The responders included a total of five patients, three of whom also received dexamethasone.

Table 4

Bortezomib-based Combination Regimens in the Treatment of Newly Diagnosed Multiple Myeloma*

Regimen	Phase	ORR†/CR (%)‡,§	Reference
Unselected patients			
Bortezomib −/+ dexamethasone	II	88%/6% (n=32)	(96)
Bortezomib + cyclophosphamide, dexamethasone	II	88%/39% (n=33)	(104)
Bortezomib + cyclophosphamide, dexamethasone	I/II	77%/10% (n=33)	(105)
Bortezomib + liposomal doxorubicin, dexamethasone	II	93%/43% (n=40)	(100, 101)
Bortezomib + lenalidomide, dexamethasone	I/II	100%/45% (n=68)	(103)
Bortezomib + thalidomide, cyclophosphamide, dexamethasone	II	96%/35% (n=44)	(214)
Bortezomib + lenalidomide, cyclophosphamide, dexamethasone	I/II	100%/56% (n=25)	(215)
Transplant-eligible patients			
Bortezomib + dexamethasone	II	66%/21% (n=48)	(97)
Bortezomib + dexamethasone vs. VAD	III	82%/15% (n=214) 65%/7% (n=210)	(108)
Bortezomib alternating with dexamethasone	II	65%/13% (n=40)	(216)
Bortezomib + infusional doxorubicin, dexamethasone (PAD)	I/II	95%/24% (n=21)	(98, 99)
Bortezomib + doxorubicin, dexamethasone vs. VAD	III	83%/5% (n=150) 59%/1% (n=150)	(217)
Bortezomib + thalidomide, dexamethasone	Pilot	87%/16% (n=33)	(102)
Bortezomib + thalidomide, dexamethasone vs. Thalidomide + dexamethasone	III	94%/32% (n=226) 79%/12% (n=234)	(109)
Bortezomib + VBMCP/VBAD vs. Bortezomib + thalidomide, dexamethasone vs. Thalidomide + dexamethasone	III	72%/28% (n=64) 80%/41% (n=56) 66%/12% (n=63)	(218)
Bortezomib + DT-PACE	I	83%/NR (n=12)	(106)
Bortezomib + Total Therapy III	II	NR/83%! (n=303)	(107)
Transplant-ineligible patients			
Bortezomib + melphalan, prednisone	I/II	89%/43% (n=60)	(110, 111)
Bortezomib + melphalan, ascorbic acid	II	74%/16% (n=35)	(219)
Bortezomib + melphalan, prednisone vs. Melphalan, prednisone	III	71%/30% (n=337) 35%/4% (n=331)	(114)
Bortezomib + melphalan, prednisone vs. Bortezomib + prednisone, thalidomide	III	81%/41% (n=98) 81%/37% (n=108)	(112)
Bortezomib + melphalan, prednisone vs. Bortezomib + melphalan, prednisone, thalidomide	III	82%/21% (n=177) 87%/39% (n=177)	(113)

*Due to space limitations, the authors have chosen to emphasize those studies that have appeared in peer-reviewed format, or phase III trials that have been presented as abstracts. We apologize in advance to our many colleagues who have reported excellent work from phase I/II studies in abstract form that has not yet been published which was omitted.

†Abbreviations: CR, complete response rate; DT-PACE, dexamethasone, continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide with daily thalidomide; NR, not reported; ORR, overall response rate; PAD, bortezomib with doxorubicin and dexamethasone; TD, thalidomide and dexamethasone; VAD, vincristine with doxorubicin and dexamethasone; VBAD, vincristine with BCNU, Adriamycin, and dexamethasone; VBMCP, vincristine with BCNU, melphalan, cyclophosphamide, and prednisone.

[‡]Response rates shown are overall responses, including complete + partial responses. Complete response rates shown incorporate CR and near-CR, where these were reported.

[§]The study evaluable population is indicated in parentheses.

[!]Data are at 24 months after the entire Total Therapy III treatment program.

Table 5

Retreatment Using Bortezomib in Patients Previously Exposed to Bortezomib-based Therapy for Multiple Myeloma*

Regimen	Study Type	Initial Response Rate	Retreatment Response Rate[‡,§]	Reference
Bortezomib	Prospective	100%	33% (n=6)	(220)
Bortezomib + dexamethasone	Retrospective	100%	100% (n=3)	(221)
Bortezomib −/+ dexamethasone	Phase IV	44%	50% (n=10)	(222)
Bortezomib −/+ dexamethasone	Retrospective	68%	50% (n=22)	(133)
Bortezomib −/+ dexamethasone	Retrospective	100%	63% (n=60)	(223)
Bortezomib −/+ dexamethasone	Retrospective	59%	22% (n=82)	(134)
Bortezomib −/+ dexamethasone	Phase II	100%	27% (n=97)	(224)
Bortezomib −/+ dexamethasone −/+ melphalan/ prednisone	Phase II	NR[†]	73% (n=47)	(135)

[†]Abbreviations: NR, not reported

[‡]Response rates shown are overall responses, including complete + partial responses.

[§]The study enrollment is indicated in parentheses.

Table 6

Ongoing Randomized Phase II and Phase III Trials of Bortezomib-based Therapy for Multiple Myeloma*

Regimen	Study Type
Relapsed and/or refractory multiple myeloma	
Bortezomib + dexamethasone vs. thalidomide + dexamethasone	NMSG‡
Bortezomib + melphalan + dexamethasone vs. bortezomib + thalidomide + dexamethasone for patients relapsing after Total Therapy II	University of Arkansas
Bortezomib + CNTO 328 vs. bortezomib + placebo	Industry-sponsored
Bortezomib + vorinostat vs. bortezomib + placebo	Industry-sponsored
Bortezomib intravenously vs. bortezomib subcutaneously	Industry-sponsored
Newly diagnosed multiple myeloma	
Bortezomib + thalidomide + dexamethasone vs. thalidomide + dexamethasone	EBMT
Bortezomib + lenalidomide + dexamethasone vs. bortezomib + dexamethasone	ECOG
Bortezomib + VBMCP/VBAD vs. thalidomide + dexamethasone vs. bortezomib + thalidomide + dexamethasone	PETHEMA
Bortezomib + melphalan + prednisone vs. bortezomib + thalidomide + prednisone; subsequent randomization to maintenance with bortezomib + thalidomide vs. bortezomib + prednisone	PETHEMA
Bortezomib + lenalidomide + dexamethasone vs. lenalidomide + dexamethasone	SWOG
Bortezomib + thalidomide + dexamethasone + D-PACE with tandem transplantation vs. tandem transplantation with D-PACE	University of Arkansas
Total Therapy III incorporating bortezomib + DT-PACE vs. Total Therapy III-Lite with one cycle of bortezomib + DT-PACE for low risk myeloma patients	University of Arkansas
Bortezomib + dexamethasone vs. bortezomib + thalidomide + dexamethasone vs. bortezomib + melphalan + prednisone	Industry-sponsored
Bortezomib + lenalidomide + dexamethasone vs. bortezomib + lenalidomide + dexamethasone + cyclophosphamide vs. bortezomib + cyclophosphamide + dexamethasone	Industry-sponsored
Consolidation	
Bortezomib after stem cell transplantation vs. no consolidation	NMSG
Bortezomib after stem cell transplantation vs. no consolidation	Industry-sponsored
Maintenance	
Bortezomib vs. observation for patients who remain event-free after Total Therapy II	University of Arkansas
Bortezomib after stem cell transplantation vs. no consolidation	Industry-sponsored

*Source: http://www.clinicaltrials.gov accessed on May 2, 2009.

‡Abbreviations: D-PACE, dexamethasone, continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide; DT-PACE, D-PACE with daily thalidomide; EBMT, European Group for Blood & Marrow Transplantation; ECOG, Eastern Cooperative Oncology Group; NMSG, Nordic Myeloma Study Group; PETHEMA, Programa para el Estudio de la Terapéutica en Hemopatía Maligna; SWOG, Southwest Oncology Group; VBAD, vincristine with BCNU, Adriamycin, and dexamethasone; VBMCP, vincristine with BCNU, melphalan, cyclophosphamide, and prednisone.