Chapter 7
Novel Proteasome Inhibitors

Robert Z. Orlowski

7.1 Introduction

The initial regulatory approval of the first-in-class proteasome inhibitor bortezomib for relapsed/refractory multiple myeloma based on data from phase I [1] and II [2] trials showing antitumor activity validated the proteasome as a rational target for cancer therapy. This was followed later by additional approvals, both as a single agent [3] and with liposomal doxorubicin [4], for relapsed disease, and with melphalan and prednisone for previously untreated symptomatic patients with myeloma [5]. Proteasome inhibitors exert their anti-myeloma effects through a number of molecular mechanisms, given the role of the proteasome in turnover of the majority of cellular proteins [6]. Among the more prominent include stabilization of proapoptotic B cell CLL/lymphoma (Bcl)-2 homology 3 (BH3) proteins and cleavage of antiapoptotic Bcl-2 and myeloid cell leukemia sequence (Mcl)-1, accumulation of cyclin-dependent kinase inhibitors resulting in cell cycle arrest, induction of stress-response pathways such as c-Jun-N-terminal kinase (JNK) and the unfolded protein response (UPR), and inhibition of nuclear factor kappa B (NF-κB) signaling, as detailed in several recent reviews [7–9]. The successful translation of bortezomib from the bench to the bedside spurred interest in the development of novel inhibitors that might have attractive properties which could be different from this first-generation agent. Broadly speaking, these agents can be divided into those that, like CEP-18770 and MLN9708, bind the proteasome reversibly and those like carfilzomib

R.Z. Orlowski (✉)
The Department of Lymphoma & Myeloma, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 429, Houston, TX 77030, USA

Department of Experimental Therapeutics, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Unit 429, Houston, TX 77030, USA
e-mail: rorlowsk@mdanderson.org

N.C. Munshi and K.C. Anderson (eds.), *Advances in Biology and Therapy of Multiple Myeloma: Volume 2: Translational and Clinical Research*,
DOI 10.1007/978-1-4614-5260-7_7,

and marizomib that bind catalytic subunits in an irreversible manner. Also, inhibitors such as bortezomib and carfilzomib seem to bind relatively indiscriminately to all isoforms of the proteasome. In contrast, some agents in development are able to bind more specifically to the so-called immunoproteasome, which is expressed to a large extent in both normal and malignant hematopoietic tissues, but not in other organs. Finally, intriguing data are emerging about the potential utility of dual proteasome inhibition with combinations of more than one inhibitor, providing students of the field and investigators with a broad range of studies to be completed before the full utility of the "zomib" class of drugs in multiple myeloma is defined.

7.2 Irreversible Proteasome Inhibitors

The first-in-class proteasome inhibitor bortezomib is a slow-binding and reversible agent [10, 11], which allows recovery of cellular proteasome activity through a number of mechanisms, including new proteasome synthesis, drug metabolism, and release of its intended target. While boronic acid peptides have the benefits of enhanced potency and specificity compared to the traditional peptide aldehydes used as laboratory probes of proteasome function, other chemistries can provide similar properties. Among these are several that bind the proteasome and form irreversible bonds, which have the theoretical advantage of providing a longer-lasting target inhibition and possibly therefore greater therapeutic efficacy. Examples of these include peptide epoxyketones such as carfilzomib, previously known as PR-171, and also lactacystin and related agents, such as marizomib, previously known as NPI-0052. Both of these drugs have been validated in preclinical studies and are now undergoing clinical trials, and other irreversible inhibitors are showing promise as well.

7.2.1 Carfilzomib

7.2.1.1 Biological Basis

Carfilzomib is a peptide epoxyketone related to epoxomicin which, like marizomib, was originally isolated from a bacterium and is currently being developed by Onyx Pharmaceuticals (Emeryville, CA). In the case of epoxomicin, the bacteria of origin was the actinomycete strain No. Q996-17 [12]. Later, epoxomicin was synthesized chemically and shown to have potent anti-inflammatory and antiproliferative effects due to its ability to induce proteasome inhibition [13–15]. Studies in models of multiple myeloma showed that low doses of this agent specifically bound the β5 constitutive proteasome and, to some extent, also the $\beta5_i$ immunoproteasome

Table 7.1 Comparison of the ability of bortezomib, marizomib, and carfilzomib to inhibit the proteolytic activities of the 20S proteasome[a]

Proteasome activity	Bortezomib	Marizomib	Carfilzomib
Trypsin-like	4,200 nM	28 nM	3,600 nM
Caspase-like	74 nM	430 nM	2,400 nM
Chymotrypsin-like	7 nM	3.5 nM	6 nM

[a]Data represent the concentration needed to reduce the indicated constitutive proteasome activity by 50% and are derived from reference [17]. Please note that, due to the use of different assays and conditions, data from Tables 7.1, 7.4, and 7.5 are not strictly comparable

subunit and inhibited their chymotrypsin-like (ChT-L) activities [16]. However, at higher concentrations of carfilzomib, binding and inhibition were also seen of the trypsin-like (T-L) activity and also the post-glutamyl peptide hydrolyzing (PGPH) or caspase-like (C-L) activity. Carfilzomib induced accumulation of ubiquitin-protein conjugates and proteasome substrates such as Bax and induced apoptosis through dual activation of caspases-8 and −9, along with the downstream effector caspase-3. This was accompanied by mitochondrial membrane depolarization with release of cytochrome c and second mitochondria-derived activator of caspases (Smac), as well as activation of JNK. Notably, carfilzomib activated caspases and programmed cell death to a greater extent than was the case for bortezomib in both myeloma cell lines and primary samples. Furthermore, carfilzomib overcame drug resistance to both conventional agents and also to bortezomib in these model systems, providing a strong rationale for its translation into the clinic.

Biochemical characterization of carfilzomib supported these in cellulo studies and showed that this agent inhibited the ChT-L activity with comparable potency to that of bortezomib but was a weaker inhibitor of the PGPH function, while both were poor inhibitors of the T-L activity (Table 7.1) [17, 18]. These studies showed that carfilzomib was able to reduce tumor cell viability with equal to greater potency than bortezomib in experiments with continuous exposure to either drug. Interestingly, when both were given as a pulse followed by a washout, to somewhat mimic what might be expected based on in vivo pharmacokinetics, carfilzomib proved superior in myeloma models, as well as cell lines representing other hematologic malignancies and solid tumors. Systemic administration of radioactive drug in animal models induced proteasome inhibition in virtually all tissues tested with the exception of the brain, and drug accumulation was seen in the adrenals, bone marrow, intestine, liver, lung, and urine. Whereas bortezomib could not be dosed on consecutive days in animal models [10, 11], carfilzomib was tolerated either on a schedule of 5 days daily or on a schedule of two consecutive days in each week [17]. The latter regimen showed enhanced antitumor efficacy in murine xenograft models of HT-29 colorectal adenocarcinoma and HS-Sultan lymphoma cells compared to bortezomib. Notably, correlative studies revealed that carfilzomib provided greater tumor tissue proteasome inhibition in these xenografts, possibly accounting for the greater activity.

Carfilzomib is active not just alone but also seems able to induce chemosensitization and overcome drug resistance. In combination with dexamethasone, for example, carfilzomib showed strongly synergistic anti-myeloma activity [16]. Inhibition of antiapoptotic Bcl-2 family members including Bcl-2, Bcl-x_L, Bcl-w, and Mcl-1 appears also to be a rational strategy with carfilzomib. Using either ABT-737 [19] or AT-101 [20], the activity of carfilzomib was potentiated against models of mantle cell lymphoma, diffuse large B cell lymphoma, and chronic lymphocytic leukemia. Suppression of histone deacetylases with agents such as vorinostat also has been shown to be synergistic with carfilzomib in diffuse large B cell lymphomas, including both germinal-center B cell-like and activated B cell-like models [21]. Finally, an additional attractive approach may be to first induce cell cycle arrest through the use of a cyclin-dependent kinase (CDK)-4/6 inhibitor, such as PD 0332991, which sensitizes cells to later cytotoxic agents including carfilzomib [22], due at least in part to loss of interferon regulatory factor 4 [23].

7.2.1.2 Clinical Development

Carfilzomib as a Single Agent

Preclinical studies with carfilzomib validated consecutive-day dosing with this agent for either 2 or 5 days as being tolerable, and these schedules were therefore translated into the clinic into two phase I trials for patients with hematologic malignancies. The first study, PX-171-001, administered carfilzomib as an intravenous push on days one through five, followed by 9 days off in every 14-day cycle, at doses ranging from 1.2 to 20 mg/m^2 [24]. Adverse events seen in at least 20% of the 29 patients treated included fatigue, nausea, diarrhea, cough, dyspnea, hypoesthesia, pyrexia, headache, peripheral edema, constipation, exertional dyspnea, and paresthesias. These rarely reached grade 3 or 4 severity, with only dyspnea and thrombocytopenia being seen in more than one patient. Dose-limiting toxicities (DLTs) in the 20 mg/m^2 cohort included one episode of grade 3 febrile neutropenia requiring hospitalization and one of grade 4 thrombocytopenia. Pharmacodynamic studies showed a carfilzomib dose-dependent inhibition of the 20S proteasome in peripheral blood mononuclear cells (PBMCs) and in whole blood. This inhibition exceeded 75% after single doses of at least 15 mg/m^2 and reached levels above 90% after the fifth consecutive dose, though these generally returned to baseline in PBMCs during the 9-day rest period. Evidence of antitumor activity was seen in one patient with mantle cell lymphoma who achieved an unconfirmed complete remission (CR), one patient with Waldenström macroglobulinemia who experienced a minor response (MR), and two patients with multiple myeloma, including one MR and one PR. Notably, the latter was in a patient with previously bortezomib-refractory disease, corroborating in part the preclinical data [16]. Importantly, peripheral neuropathy was not seen at the grade 3 or 4 level in any patients, possibly due to the greater specificity of carfilzomib for the proteasome over other targets compared to bortezomib [25].

A different schedule, with twice-weekly dosing for 3 weeks out of 4, which led to drug administration at doses ranging from 1.2 to 27 mg/m^2 on days 1, 2, 8, 9, 15, and 16 of every 28-day cycle, was evaluated in the second phase I study of carfilzomib, PX-171-002 (ClinicalTrials.gov Identifier NCT00150462)[26]. Dose-limiting toxicities occurred at 27 mg/m^2 and included an episode of hypoxia and also grade 4 thrombocytopenia. In addition, though this did not reach criteria for a DLT, reversible elevations in the serum creatinine were seen in three of five myeloma patients treated at 27 mg/m^2, which in at least some patients seemed to be associated with a rapid decline in monoclonal protein levels and possible tumor lysis. The minimal effective dose was defined as 15 mg/m^2, and among 16 patients who received dosing at this level or higher, five responses were seen, including four PRs and one MR in myeloma patients, while another two had stable disease. Some of these responses also were in previously bortezomib-refractory disease, and response durability ranged from 134 to 392 days. Since the aforementioned episodes of renal insufficiency tended to not recur with drug rechallenge, this study was later amended to allow for a lower initial dose level at 20 mg/m^2 to be given during cycle 1 and a higher dose of 27 mg/m^2 to be given during subsequent cycles. This has been reported to be well tolerated and to show evidence suggesting the possibility of an enhanced antitumor activity, though these data have not yet appeared in a peer-reviewed format.

Successful completion of the phase I studies of carfilzomib was followed by two phase II studies specifically targeting patients with multiple myeloma. The first of these, PX-171-003 (NCT00511238), enrolled patients with relapsed and refractory disease utilizing the day 1, 2, 8, 9, 15, and 16 schedule, which has been the regimen taken forward in most of the phase II and phase I combination studies. Patients also later received tumor lysis prophylaxis in the form of allopurinol and intravenous hydration, as well as a low, 4-mg dose of dexamethasone during cycle 1 only to prevent a possible cytokine release syndrome [27]. These data were updated after 46 patients had been enrolled, at which time common adverse events were anemia, diarrhea, fatigue, increased creatinine, nausea, thrombocytopenia, and upper respiratory infection. Among evaluable patients, five had achieved at least a PR, with another five having an MR, for a clinical benefit ratio of 26%, including 10 out of 39 subjects, some of whom were bortezomib refractory. Median TTP was 6.2 months, while the median DOR for patients with at least an MR was 7.4 months. Another 16 patients had achieved stable disease or better for at least 6 weeks, further supporting the activity of this agent. More recently, updated data from this study have been provided in a press release [28], which reported that the final overall response rate in this trial was 24%, while the duration of response (DOR) was 7.4 months in patients with a median of five prior lines of therapy. These data could in the future form the basis for a filing with the Food and Drug Administration for accelerated approval of carfilzomib in patients who have previously received an immunomodulatory agent and bortezomib and were refractory to their last line of therapy.

The second phase II study of carfilzomib, PX-171-004 (NCT00530816), targeted patients with relapsed disease who were earlier in their course with multiple

myeloma and had received between one and three prior lines of therapy. Cohorts were enrolled for treatment who were bortezomib naïve, bortezomib-exposed but bortezomib sensitive, and bortezomib-exposed and bortezomib refractory. In a cohort of thirty-five bortezomib-treated patients, the only grade 3 or 4 adverse events seen in at least 10% of subjects were neutropenia and anemia, and only one grade 3 neuropathy was recorded [29]. An overall response rate of 18% was seen, including patients with at least a PR, showing some evidence of clinical cross-resistance between carfilzomib and bortezomib. However, 70% of patients achieved at least stable disease, and median DOR and time to progression (TTP) were a respectable 10.6 and 5.3 months, respectively. In a larger cohort of 54 patients who were bortezomib naïve, grade 3 or 4 adverse events seen in at least 10% of subjects were fatigue, pneumonia, and thrombocytopenia [30]. Among patients who received dosing with 20 mg/m^2 of carfilzomib, the overall response rate was 46%, while 53% of those who received 27 mg/m^2 starting in cycle 2 achieved at least a PR. Median DOR and TTP values were 8.4 and 7.5 months, respectively, with the latter being superior to the TTP seen with bortezomib in bortezomib-naïve patients with relapsed disease, which was 6.2 months [3].

Carfilzomib-Based Combination Regimens

The synergistic interaction between carfilzomib and dexamethasone [16], as well as the remarkable activity of the regimen of bortezomib with lenalidomide and dexamethasone in both the relapsed/refractory [31] and up-front settings [32], prompted an evaluation of carfilzomib with lenalidomide and dexamethasone. This recently completed study, PX-171-006 (NCT00603447), used the standard carfilzomib schedule along with lenalidomide at 25 mg on days one through 21 of every 28-day cycle and once weekly dexamethasone at 40 mg [33]. Common adverse events seen in at least 25% of patients included fatigue (in 45%), diarrhea (37%), neutropenia (30%), and anemia (25%), while common grade 3/4 adverse events seen in at least 5% were neutropenia (23%), thrombocytopenia (18%), and anemia (12%). As had been the case in previous studies of carfilzomib, peripheral neuropathy was not reported at the grade 3 or 4 level. An overall response rate of 66% was seen among 80 patients, including 27.5% with at least a very good PR and 6.3% with either a CR or sCR. In the cohort that received the doses selected for further study, the overall response rate was 75%, with response rates being aided by the use of carfilzomib at 20 mg/m^2 in cycle 1, followed by dosing at 27 mg/m^2 in cycle 2 and later. Responses were robust in all patient subgroups (Table 7.2), including patients with prior exposure to bortezomib, lenalidomide, or both. These encouraging findings have formed the basis for a phase III randomized study, which will compare lenalidomide with low-dose dexamethasone to carfilzomib with lenalidomide and low-dose dexamethasone in the relapsed setting (Table 7.3). If positive, as is to be hoped, this trial would provide the confirmatory data needed to support approval of carfilzomib and its use in an earlier, less refractory patient population.

Table 7.2 Response rates to the regimen of carfilzomib, lenalidomide, and dexamethasone in subgroups of patients with relapsed and relapsed/refractory multiple myeloma[a]

	Total *n*	CR/sCR n (%)	≥VGPR *n* (%)	ORR (≥PR) *n* (%)
Prior lines of therapy				
1	17	0 (0)	8 (47.1)	13 (76.5)
2	63	5 (7.9)	19 (30.2)	40 (63.5)
Types of prior therapies				
Bor	59	2 (3.4)	16 (27.1)	34 (57.6)
Len	54	2 (3.7)	15 (27.8)	32 (59.3)
Thal	34	3 (8.8)	12 (35.3)	27 (79.4)
Len or Thal	69	5 (7.2)	21 (30.4)	44 (63.8)
Len and Thal	19	0 (0)	6 (31.6)	15 (78.9)
Bor and Len	44	1 (2.3)	10 (22.7)	23 (52.3)
Bor and Thal	22	1 (4.5)	5 (22.7)	15 (68.2)
Bor, Len, and Thal	13	0 (0)	3 (23.1)	9 (69.2)
Cytogenetics				
Normal/favorable[b]	40	2 (5.0)	15 (37.5)	28 (70.0)
Poor prognosis[c]	31	3 (9.7)	9 (29.0)	17 (54.8)
Unknown	9	0 (0)	3 (33.3)	8 (88.9)
ISS stage				
Stage I	34	2 (5.9)	13 (38.2)	25 (73.5)
Stage II	31	2 (6.5)	10 (32.3)	21 (67.7)
Stage III	9	0 (0)	2 (22.2)	4 (44.4)

[a]*Abbreviations*: *Bor* bortezomib, *CR* complete remission, *ISS* International Staging System, *Len* lenalidomide, *ORR* overall response rate, *PR* partial remission, *sCR* stringent CR, *Thal* thalidomide, *VGPR* very good PR

[b]Normal/favorable cytogenetics included patients with t(11;14) or normal cytogenetics by metaphase analysis

[c]Poor prognosis cytogenetics included patients with t(4;14), t(14;16), del17p, del13q, gain 1q21, or other abnormalities by metaphase analysis

7.2.2 ONX 0912

7.2.2.1 Biological Basis

Preclinical studies with carfilzomib to determine if it was orally bioavailable unfortunately revealed that this drug did not induce inhibition of blood or target tissue proteasomes after oral administration [34, 35]. Additional screening and rational design efforts looking for smaller peptides that might be better absorbed through the gastrointestinal tract led to the identification of PR-047, which is now known as ONX 0912 (Onyx Pharmaceuticals), as a potential drug candidate. This N-capped tripeptide epoxyketone contains leucine in the P1 position, which is the residue that forms a bond with the N-terminal threonine active site of proteolytically active proteasome subunits, and methoxylated serine residues in the P2 and P3 positions, which are the next residues towards the N-terminus of the peptide, providing greater aqueous solubility. Like carfilzomib, ONX 0912 exhibited strong specificity for the

Table 7.3 Ongoing studies of carfilzomib in patients with multiple myeloma[a]

Study title	Carfilzomib dosing
A Study of Carfilzomib maintenance therapy in subjects previously enrolled in Carfilzomib treatment protocols	IV push on days 1, 2, 15, and 16 of a 28-day cycle
Multicenter, open-label, single-arm, phase 1b/2 study of the safety and efficacy of combination treatment w/ Carfilzomib, Lenalidomide, and Dexamethasone in subjects w/ newly diagnosed, previously untreated multiple myeloma requiring systemic chemotherapy	IV infusion on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle for cycles 1–8 (induction) and on days 1, 2, 15, and 16 of a 28-day cycle for cycles 9+ (maintenance)
Phase 2 Study of the safety and pharmacokinetics of Carfilzomib in subjects with relapsed and refractory multiple myeloma and varying degrees of renal function	IV on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle
Compassionate use study of Carfilzomib for patients with relapsing or resistant multiple myeloma	Carfilzomib (20 mg/m^2) IV push to be given at maximum rate of 10 ml/min on day 1 and day 2 of cycle 1 only Carfilzomib (27 mg/m^2) IV bolus to be given at maximum rate of 10 ml/min on days 8, 9, 15, and 16 of cycle 1, then through cycle 2 and beyond if initial dosing with 20 mg/m^2 tolerated For patients who tolerated 27 mg/m^2 through cycle 2 days 1 and 2, carfilzomib dose may be escalated to 36 mg/m^2 on days 8, 9, 15 and 16 of cycle 2
Phase 1b multicenter dose escalation Study of Carfilzomib with lenalidomide and dexamethasone for safety and activity in relapsed multiple myeloma	First 12 cycles, IV infusion twice weekly for 3 weeks of a 28-day cycle. Remaining 6 cycles, twice weekly during weeks 1 and 3 of a 28-day cycle
An open-label, single-arm, Phase 2 study of Carfilzomib in patients with relapsed and refractory multiple myeloma	IV push twice weekly for three weeks followed by 12 days of rest
A Phase I/II Trial of Cyclophosphamide, Carfilzomib, Thalidomide, and Dexamethasone in patients with newly diagnosed active multiple myeloma	Patients receive carfilzomib IV on days 1, 2, 8, 9, 15, and 16
A randomized, multicenter, Phase 3 study comparing Carfilzomib, Lenalidomide, and Dexamethasone vs Lenalidomide and Dexamethasone in subjects with relapsed multiple myeloma	IV on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle
Phase 1b/2, multicenter open-label study of the safety and activity of Carfilzomib in subjects with relapsed solid tumors and in multiple myeloma	IV on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle
An open-label, single-arm, Phase 2 study of Carfilzomib in patients with relapsed multiple myeloma	IV on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle

[a]Data are from a search of http://www.clinicaltrials.gov performed on October 10, 2010, using the terms “carfilzomib” and “multiple myeloma”

chymotrypsin-like proteasome activity, with an IC_{50} for the β5 subunit of 36 nM, and for the low molecular mass polypeptide (LMP)-7 immunoproteasome subunit of 82 nM [35]. Following oral dosing in rodents and dogs, proteasome inhibition in excess of 80% could be achieved in virtually all tissues examined, except for the brain, with an onset in 15 min, which was comparable to that of intravenous carfilzomib. Doses needed to achieve this level of inhibition were up to tenfold below the maximum tolerated dose and were tolerated on a daily for 5 days in a row schedule [34, 36]. Murine studies with ONX 0912 in BNX mice in vivo utilizing RL cell- and CT-26 cell-based models of human non-Hodgkin lymphoma and colorectal carcinoma, respectively, showed significant antitumor activity using oral dosing on days 1 and 2 of each week [35]. With respect to plasma cell dyscrasias, activity was also seen in an in vivo model of human multiple myeloma based on MM1.S cells [34]. Waldenström macroglobulinemia may be another attractive target, since ONX 0912 induced cytotoxicity in primary Waldenström and IgM-secreting lymphoma cell lines through proteasome inhibition, suppression of NF-κB, activation of c-Jun-N-terminal kinase, and induction of the unfolded protein response [37].

Combination regimens based on ONX 0912 have been investigated preclinically as well, and as was the case for carfilzomib, combinations with inhibitors of CDK-4/6 appear to be attractive. By using the CDK-4/6 inhibitor PD 0332991 to induce a G1 arrest, myeloma cell lines and primary cells were sensitized to ONX 0912 in a synergistic manner even in the presence of protective stromal cells. This combination was active through mitochondrial membrane depolarization and activation of caspase-9, as well as induction of proapoptotic BH3-only proteins such as Bcl-2-interacting mediator of cell death (Bim), which presumably negated the effects of antiapoptotic proteins such as Bcl-2 [22, 38]. ONX 0912 could also have promise in combination with bortezomib [37], since this regimen showed synergistic cell killing of Waldenström cells.

7.2.3 Marizomib

Salinosporamide A, later renamed NPI-0052, and more recently marizomib, was initially isolated as a metabolite of Salinispora tropica strain CNB-440, a seawater-requiring marine actinomycete [39]. This agent, which is being developed by Nereus Pharmaceuticals (San Diego, CA), is structurally related to omuralide and lactacystin [40] and can now be chemically synthesized quite efficiently through a number of approaches [41], making it accessible for large-scale preclinical and clinical studies.

7.2.3.1 Biological Basis

Initial studies of marizomib in models of multiple myeloma showed that it was able to suppress all three major proteolytic activities of the proteasome [42]. In comparison

Table 7.4 Comparison of the ability of bortezomib and marizomib to inhibit the proteolytic activities of the 20S proteasome[a]

Proteasome activity	Bortezomib	Marizomib
Trypsin-like	67 nM	2 nM
Caspase-like	10 nM	34 nM
Chymotrypsin-like	7.9 nM	3.5 nM

[a]Data represent the concentration needed to reduce the indicated proteasome activity by 50% and are derived from reference [42]. Please note that, due to the use of different assays and conditions, data from Tables 7.1, 7.4, and 7.5 are not strictly comparable

with bortezomib, it was a more potent inhibitor of the ChT-L activity in erythrocyte proteasomes and a much more potent inhibitor of the T-L activity, though a weaker inhibitor of the PGPH activity (Table 7.4). Notably, using maximally tolerated doses of both agents in an in vivo model, marizomib provided a longer duration of ChT-L activity inhibition than bortezomib and suppressed the T-L activity, whereas bortezomib actually stimulated it, though, in agreement with the in vitro data, it was a weaker inhibitor of the PGPH function. Later studies have shown that marizomib may also be able to provide a longer duration of ChT-L activity suppression in tumor tissues as compared to some other organs such as peripheral blood [43]. Functional assays showed that marizomib blocked activation of NF-κB more potently than was the case for bortezomib [42]. Indeed, other studies have documented that marizomib is not only superior to bortezomib in this regard but also compared to other proteasome inhibitors such as MG-132, ALLN, and lactacystin [44]. Also, in primary samples, it induced programmed cell death with DNA fragmentation to a greater extent, was able to overcome both adhesion- and cytokine-mediated drug resistance, and retained activity in samples from bortezomib-refractory patients [42]. Using a human plasmacytoma xenograft model, marizomib was shown to be able to significantly delay tumor growth and to prolong survival. Apoptotic induction was associated with activation of caspases-8, -9, and -3, but studies with dominant negative constructs showed a greater reliance for cell death on the caspase-8-dependent arm than was the case for bortezomib. Other mechanisms that appeared to contribute to cell death included mitochondrial release of cytochrome c and Smac, cleavage of poly-(ADP-ribose) polymerase, and activation of Bax. Finally, marizomib may have other benefits for patients with multiple myeloma, based on preclinical studies that documented its ability to inhibit tumor necrosis factor-mediated receptor activator of NF-κB ligand (RANKL)-induced osteoclastogenesis [44].

As is the case for other proteasome inhibitors, marizomib may prove most active in combination with other agents. Intriguingly, synergistic anti-myeloma activity has been seen in dexamethasone-sensitive MM1.S cells and in dexamethasone-resistant MM1.R cells, when bortezomib and marizomib were combined [42]. These latter findings were later confirmed in other multiple myeloma cell lines and in primary patient samples [45], and this regimen was found to suppress myeloma cell migration and measures of angiogenesis. Combination proteasome inhibitor therapy was more active against in vivo models of multiple myeloma and was effective

through enhanced activation of caspases and JNK, as well as increased suppression of NF-κB. Dual targeting strategies of this type have also shown promise in preclinical studies in models of Waldenström macroglobulinemia [46]. Another combination of interest for myeloma may be that of marizomib and the immunomodulatory agent lenalidomide, which have been shown to interact synergistically through induction of caspases-8, -9, -3, and -12, cleavage of poly-(ADP-ribose) polymerase, and activation of Bim [47]. These findings were also borne out in studies of in vivo models, where low-dose combinations of marizomib and lenalidomide significantly inhibited tumor growth and also prolonged survival. Finally, regimens of marizomib with inhibitors of CDK-4/6 are also intriguing, based on studies showing that G1 arrest markedly sensitized primary myeloma cells to proteasome inhibitors, including bortezomib and NPI-0052 [48].

7.2.3.2 Clinical Development

Marizomib is currently being studied predominantly in the phase I setting, with trials focusing on patients with solid tumors, as well as with hematologic malignancies, including multiple myeloma. The first-in-man study dosed marizomib once weekly for 3 weeks out of every 4-week cycle (NCT00396864) and did not initially report any dose-limiting toxicities. However, one serious adverse event was seen in the form of an episode of methicillin-resistant Staphylococcus aureus sepsis and postinfectious glomerulonephritis with renal failure, which did recover after antibiotic treatment [49]. Interestingly, preclinical studies have shown that the renal medulla and cortex are areas in which there is substantial accumulation of marizomib [43], and the previously noted episodes of renal insufficiency with carfilzomib do suggest caution in the use of irreversible inhibitors in patients with renal compromise. Later updates did not reveal any further renal adverse events, and toxicities included nausea, vomiting, diarrhea, fatigue, muscle stiffness, dizziness, headache, insomnia, hypotension, hypomagnesemia, anemia, and febrile neutropenia, with minimal thrombocytopenia or neuropathy [50, 51]. Proteasome inhibition of up to 100% was seen in peripheral whole blood, with a return to baseline within 1 week of each dose. Responses were not seen in patients with solid tumors, though stable disease was noted in patients with cervical carcinoma, as well as others with colorectal, hepatocellular, adenoid cystic, melanoma, granulosa cell, and ovarian tumors [51]. A second study using the same dosing schedule (NCT00629473)[52] has reported two DLTs, including one of dizziness and an unsteady gait, while another was described as “transient hallucinations” with “visual imprints when (the patient’s) eyes (were) closed [53].” Stable disease was seen as the best outcome in this study as well, including in patients with mantle cell and follicular non-Hodgkin lymphoma, Hodgkin lymphoma, sarcoma, prostatic adenocarcinoma, and melanoma. Later, this study was amended to include a bortezomib-like twice-weekly dosing, which produced common adverse events including fatigue, dysgeusia, reversible infusion site pain, lymphopenia, headaches, dizziness and/or unsteady gait, and changes in cognition [54]. A clinical benefit, including either stable disease

or evidence of regression, was then noted in a larger array of patients, including some with myeloma and cutaneous marginal zone lymphoma.

One study has focused exclusively on patients with relapsed and/or refractory multiple myeloma and also using the weekly for three out of every 4-week schedule (NCT00461045) [55]. Dose-limiting toxicities included fatigue and mental status changes with loss of balance, and other patients required dose reductions due to nausea and vomiting, as well as vertigo and confusion with word-finding difficulties. These toxicities have since been ameliorated with the addition of prophylactic antiemetics and with meclizine. An unconfirmed partial response was seen in one patient with IgA myeloma who was bortezomib-exposed and bortezomib sensitive, along with one minor response, and several patients had stable disease, including two who were previously bortezomib refractory.

7.3 Reversible Proteasome Inhibitors

While irreversible inhibitors have the theoretical advantage of binding and inhibiting the proteasome for an extended period of time, preclinical and clinical studies have shown that their duration of inhibition is only modestly longer than what would be expected for bortezomib [17, 18, 24, 42–44]. These findings suggest that new proteasome synthesis and/or assembly remains the predominant mechanism for recovery of proteolytic function in cells challenged with proteasome inhibitors. In the absence of a clear advantage for irreversible agents, therefore, reversible inhibitors with properties distinct from bortezomib, such as CEP-18770 and MLN9708, are moving forward in development for multiple myeloma and other malignant and even nonmalignant diseases.

7.3.1 CEP-18770

7.3.1.1 Biological Basis

CEP-18770 is being developed by Cephalon, Inc. (Frazer, PA) and is a dipeptide boronic acid which, like bortezomib, contains leucine in the P1 position. Unlike bortezomib, which has a phenylalanine in the P2 position, CEP-18770 contains threonine in this location instead [56–58], possibly reducing its hydrophobicity. Preclinical studies in myeloma models demonstrated the ability of CEP-18770 to inhibit the chymotrypsin-like proteasome activity with comparable potency to that of bortezomib. Slightly weaker inhibition was seen of the caspase-like activity by CEP-18770 than with bortezomib, while neither agent impacted on the trypsin-like function. Consistent with its ability to target the proteasome, CEP-18770 inhibited tumor necrosis factor-mediated activation of NF-κB by stabilizing IκB. It induced

apoptosis mediated by caspases-3, -7, and -9 in cell line and primary myeloma models and reduced endothelial cell survival, proliferation, and tubular morphogenesis. Also, this agent was shown to suppress macrophage colony-stimulating factor/receptor activator for NF-κB ligand-mediated osteoclastogenesis. Notably, CEP-18770 showed enhanced antitumor activity and increased levels of tumor proteasome inhibition compared to bortezomib in an in vivo myeloma model when both agents were administered intravenously. This occurred in conjunction with reduced cytotoxic effects on bone marrow stromal cells and a lesser impact on colony formation by bone marrow progenitor cells of both myeloid and erythroid lineages [57], suggesting the possibility that it may have a superior therapeutic index. More recent studies have shown that CEP-18770 could be combined with melphalan or bortezomib to induce synergistic anti-myeloma activity in vitro, that it could overcome either melphalan- or bortezomib-resistant tumors in vivo, and that it was effective with oral dosing [59].

7.3.1.2 Clinical Development

These encouraging preclinical findings have supported the translation of CEP-18770 to the clinic. One phase I study that administered CEP-18770 as an intravenous infusion on days 1, 4, 8, and 11 of every 21-day cycle to patients with solid tumors and non-Hodgkin lymphoma was completed in Europe (NCT00572637), but results of this trial have not yet been reported. A second, international phase I/II study to evaluate the safety and efficacy of CEP-18770 given intravenously on days 1, 8, and 15 of every 28-day cycle is currently underway in patients with relapsed and refractory multiple myeloma (NCT01023880). Finally, a combination study of CEP-18770 with lenalidomide and dexamethasone is being planned as well. All of these will be following the pharmacokinetics of CEP-18770 using a novel, high-pressure liquid chromatography/mass spectrometry-based technique to determine plasma drug levels [60].

7.3.2 MLN9708

7.3.2.1 Biological Basis

The first proteasome inhibitor to have reached the clinic in an oral formulation is MLN9708, which is being developed by Millennium: The Takeda Oncology Company (Cambridge, MA). This dipeptide has leucine in the P1 position and glycine in the P2 position and is a prodrug with a protected cyclic boron that is hydrolyzed to the active boronic acid, MLN2238, upon exposure to aqueous solutions or plasma [61]. MLN2238 preferentially bound to the β5 constitutive proteasome subunit with comparable potency to that of bortezomib (IC_{50} 3.4 nM/L for the former

Table 7.5 Comparison of the ability of bortezomib and MLN2238 to inhibit the proteolytic subunits of the 20S proteasome[a]

Proteasome activity	Bortezomib	MLN2238
Trypsin-like	1,200 nM	3,500 nM
Caspase-like	24 nM	31 nM
Chymotrypsin-like	2.4 nM	3.4 nM

[a]Data represent the concentration needed to reduce the indicated proteasome activity by 50% and are derived from reference [61]. Please note that, due to the use of different assays and conditions, data from Tables 7.1, 7.4, and 7.5 are not strictly comparable

versus 2.4 for the latter), and the two showed similar abilities to inhibit activation of NF-κB in cell-based assays. Substantially weaker binding was seen to the β1 and β2 subunits in a pattern that was also similar to bortezomib, but the binding affinity seemed even weaker than was the case for its predecessor (Table 7.5). A major difference was seen in the proteasome dissociation half-life, which was 110 min for bortezomib, but only 18 min for MLN2238, suggesting the possibility of a more rapid recovery of proteasome function, which was confirmed in washout studies in cell culture models in vitro. While this may at first seem to be a disadvantageous feature, it could in fact be a strength compared to bortezomib, if MLN2238 could more rapidly dissociate from its binding sites on proteasomes in the blood and redistribute into tumor tissues bindings. Consistent with this possibility, MLN2238 showed a greater blood volume of distribution than bortezomib at steady state in in vivo studies utilizing maximum tolerated doses of each agent [61].

In xenograft models of human lymphoma and prostate cancer, MLN2238 showed comparable peak blood proteasome inhibition levels to that of bortezomib but a shorter area under the effect versus time curve. In contrast, in tumor tissue itself, treatment with MLN2238 induced a greater and more sustained level of proteasome inhibition, as well as of downstream pharmacodynamic markers, including accumulation of growth arrest DNA damage 34. Three models were evaluated for antitumor activity, including one of prostate cancer using CWR22 cells and both subcutaneous and disseminated models of lymphoma using WSU-DLCL2 and OCI-Ly7-Luc cells, respectively. Whereas bortezomib showed modest activity against the CWR22 model system, MLN2238 induced a significantly greater growth delay, and comparable findings were obtained in both lymphoma models [61]. Subsequent studies have shown the ability of MLN2238 to retain activity in a lymphoma model that was resistant to bortezomib therapy [62].

One in vivo plasma cell dyscrasia model, the double transgenic F1 hybrid iMyc^{Ca}/Bcl-x$_L$ mouse, which develops plasma cell malignances with a short onset, has also been studied to determine the activity of MLN2238. Pharmacodynamic studies showed that 83–84% proteasome inhibition was achieved in both the blood and marrow compartments [63]. Treatment with MLN9708, as well as with bortezomib, produced a reduction in tumor burden and a significant prolongation in the median tumor-free survival [63–65].

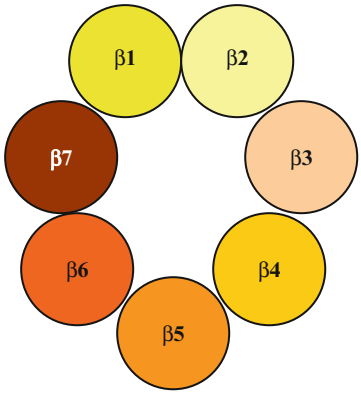
7.3.2.2 Clinical Development

Clinical trials of MLN9708 are currently underway utilizing both intravenous as well as oral dosing, and one report has been presented of a study in patients with non-hematologic malignancies [66]. In this trial (NCT00830869), patients received MLN9708 intravenously on the standard bortezomib schedule of days 1, 4, 8, and 11 of every 21-day cycle. Common adverse events have included fatigue, nausea, and pyrexia, grade 3/4 adverse events included anemia and thrombocytopenia, and dose-limiting toxicities included rash, reversible thrombocytopenia, and reversible renal failure. The available pharmacokinetic data suggested that MLN9708 showed a dose-proportional systemic exposure and a half-life of about 7 days after dosing on day 11. Moreover, reversible blood target inhibition was seen as predicted from the preclinical studies, with substantial return of proteasome function to normal within 2–4 h of dosing. A second trial evaluating MLN9708 using intravenous dosing on days 1, 8, and 15 of every 28-day cycle is targeting patients with Hodgkin and non-Hodgkin lymphoma (NCT00893464).

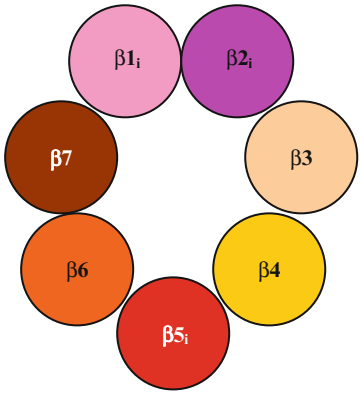
MLN9708 is also being studied in patients with relapsed and refractory multiple myeloma. One of these is a phase I trial of oral MLN9708, which is being administered on days 1, 8, and 15 of an every 28-day cycle (NCT00963820). The second is a phase I/II study using the standard bortezomib schedule of days 1, 4, 8, and 11 given every 21 days (NCT00932698). Data from these trials will hopefully be available for presentation at the 2010 meeting of the American Society of Hematology.

7.4 Immunoproteasome Inhibitors

The proteasome variant expressed in most somatic tissues is known as the constitutive proteasome, and its 20S catalytic core contains at least three subunits with proteolytic activity, known as β1, β2, and β5 (Fig. 7.1). In the presence of cytokines such as tumor necrosis factor α or γ-interferon, however, production of three alternate subunits, known as low molecular mass polypeptide (LMP)-2, multicatalytic endopeptidase complex subunit (MECL)-1, and LMP-7, or $\beta1_i$, $\beta2_i$, and $\beta5_i$, is stimulated. These subunits may be preferentially incorporated into new proteasomes under these conditions to replace β1, β2, and β5, respectively, producing a variant known as the immunoproteasome [7–9, 67]. This has been so named due to data supporting a role for its ability to generate more hydrophobic, antigenic peptides that can be presented in the context of major histocompatibility class I molecules [67]. Interestingly, cells of hematopoietic origin normally express the immunoproteasome, which may be present in conjunction with the constitutive proteasome in myeloma, while in some cases it is the predominant isoform found in primary plasma cells [18, 68]. Given the restricted, tissue-specific expression of the immunoproteasome, it may represent a target for myeloma therapy whose inhibition could provide an enhanced therapeutic index due to the paucity of expression in neural and gastrointestinal tissues. Immunoproteasome-specific inhibitors could therefore



Major Proteolytically Active Subunits of the Constitutive Proteasome	
β1	Post-glutamyl peptide hydrolyzing, or caspase-like activity
β2	Trypsin-like activity
β5	Chymotrypsin-like activity



Major Proteolytically Active Subunits of the Immunoproteasome	
β1i, also known as low molecular mass polypeptide (LMP)-2	Post-glutamyl peptide hydrolyzing, or caspase-like activity
β2i, also known as multicatalytic endopeptidase complex-like (MECL)-1	Trypsin-like activity
β5i, also known as LMP-7	Chymotrypsin-like activity

Fig. 7.1 *Proteasome variants that have been validated as targets for multiple myeloma*. The constitutive proteasome is present in most tissues in the body and consists of a barrel-shaped structure with four rings surrounding a central pore. Each of the inner two rings contains seven unique β subunits, three of which encode the major proteolytic activities of the proteasome. A cross section through one of these rings of the constitutive proteasome is shown in the *top panel*, whereas the *bottom panel* shows a comparable cross section through the immunoproteasome

target the proteasome specifically in hematologic malignancies, unlike agents such as bortezomib and carfilzomib, which do not discriminate between the constitutive and immunoproteasomes.

7.4.1 Peptide Aldehyde Inhibitors

The first immunoproteasome-specific inhibitor (IPSI) developed and tested against models of multiple myeloma was IPSI-001, a dipeptide aldehyde with norleucine in the P1 position and leucine in the P2 position [68]. Screening efforts using purified

constitutive and immunoproteasome preparations suggested that amino acid residues with greater hydrophobic character in the P1 position, such as norleucine or phenylalanine, provided a measure of immunoproteasome specificity. Consistent with this hypothesis, IPSI-001 showed a more than 100-fold increased potency to inhibit the chymotrypsin-like and branched chain amino acid preferring activities of the immunoproteasome over the constitutive proteasome. This agent bound specifically to the $\beta1_i$ subunit both in vitro and in cellulo, which was to some extent unexpected, since $\beta5_i$ contains the chymotrypsin-like activity, suggesting that binding to $\beta1_i$ caused an allosteric shift that precluded substrate entry into the $\beta5_i$ binding site. IPSI-001 induced accumulation of ubiquitin-protein conjugates, including ubiquitinated IκBα, proteasome substrates such as p21, and activated Bax as well as c-Jun-N-terminal kinase. These effects in part contributed to stimulation of programmed cell death through both caspase-8- and caspase-9-mediated pathways, resulting in dual downstream activation of the effector caspase-3. Notably, these effects were preferentially seen in immunoproteasome-expressing model systems, while those expressing the constitutive proteasome were relatively spared. Importantly, IPSI-001 was able to induce cell death in patient-derived plasma cells and in primary cells from patients with other hematologic malignancies. Also, IPSI-001 overcame drug-resistant phenotypes and was even active in primary samples from patient with clinically bortezomib-refractory disease. Further studies of IPSI-001 and other related peptide aldehydes with specificity for the immunoproteasome will, however, remain restricted to the preclinical arena, since these agents do not have sufficient potency and in vivo stability to warrant clinical application.

7.4.2 Ketoepoxide Inhibitors

Immunoproteasome-specific inhibitors with enhanced potency have been developed based on the ketoepoxide pharmacophore, which may prove to be more clinically relevant. The first of these was PR-957 (Onyx Pharmaceuticals) which, like IPSI-001, was shown to target the chymotrypsin-like proteasome activity [69]. However, unlike IPSI-001, which bound to $\beta1_i$, PR-957 bound specifically to LMP-7, or $\beta5_i$, demonstrating the ability of a directly binding agent to inhibit the chymotrypsin-like activity of the proteasome. While this drug has not been tested against multiple myeloma, it did show the ability to block inflammatory cytokine production from mononuclear cells and attenuated progression of experimental arthritis in animal models. Of potential interest to the myeloma field was its ability to reduce production of interleukin-6, which plays a role in myeloma pathobiology [70–72], as well as in resistance to drugs such as bortezomib [73] and dexamethasone [74].

A second ketoepoxide immunoproteasome-specific inhibitor that has been studied in models of multiple myeloma is PR-924 [75]. This agent was also found to be LMP-7 selective and exerted antiproliferative and proapoptotic effects with drug concentrations in the micromolar range. PR-924 was able to overcome resistance to standard chemotherapeutics such as dexamethasone, doxorubicin, and melphalan

and also subverted resistance due to cell-mediated adhesion to stroma, as well as resistance due to cytokines such as interleukin-6. At the molecular level, PR-924 activated caspases-8, -9, and -3, reduced levels of antiapoptotic Bcl-2, induced cleavage of (BH3-interacting domain death agonist) Bid to tBid, and caused loss of the normal trans-mitochondrial membrane potential with migration of cytochrome c into the cytoplasm. Finally, PR-924 was active against myeloma in both a severe combined immunodeficiency-hu model and a human plasmacytoma xenograft model.

While the data with IPSI-001 and PR-924 provide a strong rationale for translation of immunoproteasome-specific agents to the clinic to fight multiple myeloma, it should be noted that one study has suggested that inhibiting the immunoproteasome alone is not sufficient to induce cytotoxicity. Using a different specific ketoepoxide compound, these investigators found that, in MM1.S myeloma cells, inhibition of either the constitutive proteasome alone or the immunoproteasome alone did not reduce cell viability [18]. Only when these agents were combined, or when carfilzomib was used, which inhibits both proteasome variants, was there substantial cytotoxic activity. Moreover, these findings were paralleled by the effects of these agents on intracellular accumulation of ubiquitin-protein conjugates, which were marginal with either specific inhibitor alone but substantial with the combination or carfilzomib. Further preclinical studies seem therefore to be in order to validate the potential of immunoproteasome inhibitors before their translation into the clinic.

7.5 Conclusions

Second-generation, novel proteasome inhibitors are making significant progress both preclinically and clinically along the drug development path leading to regulatory approvals. Among irreversible inhibitors, carfilzomib and marizomib, which may bind the proteasome more exclusively than other proteases or with broader specificity compared to bortezomib, respectively, have already reached the clinic. Carfilzomib has shown activity in relapsed and relapsed/refractory myeloma, and though there is evidence for cross-resistance in patients with prior bortezomib therapy, it appears to have a more favorable toxicity profile, especially in regard to peripheral neuropathy. A combination regimen with lenalidomide and dexamethasone has also shown encouraging tolerability and activity, and regimens using higher doses of carfilzomib starting with the second cycle may enhance the efficacy of this agent further. Marizomib has also shown activity against multiple myeloma in a smaller number of studies, and, while like carfilzomib it does not appear to confer a significant risk of peripheral neuropathy, other neurologic effects have been noted. Interestingly, unlike bortezomib, which can be safely given in patients with renal failure without dose adjustments [76–78], early phase studies of both carfilzomib and marizomib have documented rare episodes of treatment-emergent renal insufficiency. This suggests that formal studies of these agents in patients with renal impairment will be needed, and indeed one such study with carfilzomib is already underway (NCT00721734).

Reversible inhibitors are being developed as well, including novel boronic acids that can be delivered either intravenously or orally, and which may have superior pharmacokinetics to bortezomib. The latter agents, if ultimately approved, could also more easily be used in settings such as maintenance after either standard-dose induction therapy or after high-dose therapy with autologous stem cell transplantation. More targeted agents that suppress only the immunoproteasome may have a role to play, though there is disagreement in the literature as to whether immunoproteasome inhibition is by itself sufficient to induce programmed cell death in models of multiple myeloma. Finally, and perhaps most intriguingly, combination regimens with more than one proteasome inhibitor have shown enhanced preclinical activity. If similar synergy were seen clinically, these agents could possibly be used at lower doses to achieve the same or even a superior antitumor efficacy, with the potential for a much reduced toxicity profile. Taken together, these findings strongly argue that proteasome inhibitors will not only remain part of our arsenal against multiple myeloma but will probably play an ever increasing role in our armamentarium against this disease.

Abbreviations

Bax	Bcl-2-associated X protein
Bcl-2	B cell CLL/lymphoma-2
BH3	Bcl-2 homology 3
Bid	BH3-interacting domain death agonist
BIM	Bcl-2-interacting mediator of cell death
Bor	Bortezomib
CDK	Cyclin-dependent kinase
ChT-L	Chymotrypsin-like
C-L	Caspase-like
CR	Complete remission
DLT	Dose-limiting toxicity
DOR	Duration of response
IκB	Inhibitor of nuclear factor kappa B
IPSI	Immunoproteasome-specific inhibitor
ISS	International Staging System
JNK	c-Jun-N-terminal kinase
Len	Lenalidomide
LMP	Low molecular mass polypeptide
Mcl-1	Myeloid cell leukemia sequence 1
MECL	Multicatalytic endopeptidase complex-like
MR	Minor response
MTD	Maximum tolerated dose
NF-κB	Nuclear factor kappa B
ORR	Overall response rate

PBMCs	Peripheral blood mononuclear cells
PGPH	Post-glutamyl peptide hydrolyzing also referred to as the caspase-like (C-L) activity
PR	Partial remission
RANKL	Tumor necrosis factor-mediated receptor activator of NF-κB ligand
sCR	Stringent CR
Smac	Second mitochondria-derived activator of caspases
T-L	Trypsin-like
Thal	Thalidomide
TTP	Time to progression
UPR	Unfolded protein response
VGPR	Very good PR

Acknowledgements The author would like to acknowledge research support from the National Cancer Institute (P50-CA-142509, P01-CA-124787, R01-CA-102278, R01-CA-134786), as well as the Multiple Myeloma Research Foundation and The Leukemia & Lymphoma Society.

References

1. Orlowski RZ, Stinchcombe TE, Mitchell BS et al (2002) Phase I trial of the proteasome inhibitor PS-341 in patients with refractory hematologic malignancies. J Clin Oncol 20(22):4420–7
2. Richardson PG, Barlogie B, Berenson J et al (2003) A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med 348(26):2609–17
3. Richardson PG, Sonneveld P, Schuster MW et al (2005) Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med 352(24):2487–98
4. Orlowski RZ, Nagler A, Sonneveld P et al (2007) Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: combination therapy improves time to progression. J Clin Oncol 25(25):3892–901
5. San Miguel JF, Schlag R, Khuageva NK et al (2008) Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med 359(9):906–17
6. Rock KL, Gramm C, Rothstein L et al (1994) Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. Cell 78:761–71
7. Orlowski RZ, Kuhn DJ (2008) Proteasome inhibitors in cancer therapy: lessons from the first decade. Clin Cancer Res 14(6):1649–57
8. Shah JJ, Orlowski RZ (2009) Proteasome inhibitors in the treatment of multiple myeloma. Leukemia 23(11):1964–79
9. Dick LR, Fleming PE (2010) Building on bortezomib: second-generation proteasome inhibitors as anti-cancer therapy. Drug Discov Today 15(5–6):243–9
10. Adams J, Behnke M, Chen S et al (1998) Potent and selective inhibitors of the proteasome: dipeptidyl boronic acids. Bioorg Med Chem Lett 8(4):333–8
11. Adams J, Palombella VJ, Sausville EA et al (1999) Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 59(11):2615–22
12. Hanada M, Sugawara K, Kaneta K et al (1992) Epoxomicin, a new antitumor agent of microbial origin. J Antibiot 45(11):1746–52
13. Sin N, Kim KB, Elofsson M et al (1999) Total synthesis of the potent proteasome inhibitor epoxomicin: a useful tool for understanding proteasome biology. Bioorg Med Chem Lett 9:2283–8

14. Meng L, Mohan R, Kwok BH, Elofsson M, Sin N, Crews CM (1999) Epoxomicin, a potent and selective proteasome inhibitor, exhibits in vivo antiinflammatory activity. Proc Natl Acad Sci USA 96(18):10403–8
15. Kim KB, Myung J, Sin N, Crews CM (1999) Proteasome inhibition by the natural products epoxomicin and dihydroeponemycin: insights into specificity and potency. Bioorg Med Chem Lett 9(23):3335–40
16. Kuhn DJ, Chen Q, Voorhees PM et al (2007) Potent activity of carfilzomib, a novel, irreversible inhibitor of the ubiquitin-proteasome pathway, against preclinical models of multiple myeloma. Blood 110(9):3281–90
17. Demo SD, Kirk CJ, Aujay MA et al (2007) Antitumor activity of PR-171, a novel irreversible inhibitor of the proteasome. Cancer Res 67(13):6383–91
18. Parlati F, Lee SJ, Aujay M et al (2009) Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. Blood 114(16):3439–47
19. Paoluzzi L, Gonen M, Bhagat G et al (2008) The BH3-only mimetic ABT-737 synergizes the antineoplastic activity of proteasome inhibitors in lymphoid malignancies. Blood 112(7): 2906–16
20. Paoluzzi L, Gonen M, Gardner JR et al (2008) Targeting Bcl-2 family members with the BH3 mimetic AT-101 markedly enhances the therapeutic effects of chemotherapeutic agents in in vitro and in vivo models of B-cell lymphoma. Blood 111(11):5350–8
21. Dasmahapatra G, Lembersky D, Kramer L et al (2010) The pan-HDAC inhibitor vorinostat potentiates the activity of the proteasome inhibitor carfilzomib in human DLBCL cells in vitro and in vivo. Blood 115(22):4478–87
22. DiLiberto M, Huang X, Zewdu R et al (2009) Selective inhibition of CDK4/CDK6 sensitizes bone marrow myeloma cells for killing by proteasome inhibitors carfilzomib and PR-047 through cell cycle-dependent expression of pro-apoptotic Noxa and Bim. Blood (ASH Annual Meeting Abstract) 114:2854
23. Huang X, Di Liberto M, Ely S et al (2009) Induction of sequential G1 arrest and synchronous S phase entry by reversible CDK4/CDK6 inhibition sensitizes myeloma cells for cytotoxic killing through loss of IRF-4. Blood (ASH Annual Meeting Abstract) 114:299
24. O'Connor OA, Stewart AK, Vallone M et al (2009) A phase 1 dose escalation study of the safety and pharmacokinetics of the novel proteasome inhibitor carfilzomib (PR-171) in patients with hematologic malignancies. Clin Cancer Res 15(22):7085–91
25. Arastu-Kapur S, Shenk K, Parlati F, Bennett MK (2008) Non-proteasomal targets of proteasome inhibitors bortezomib and carfilzomib. Blood (ASH Annual Meeting Abstract) 112:2657
26. Alsina M, Trudel S, Vallone M, Molineaux C, Kunkel L, Goy A (2007) Phase 1 single agent antitumor activity of twice weekly consecutive day dosing of the proteasome inhibitor carfilzomib (PR-171) in hematologic malignancies. Blood (ASH Annual Meeting Abstract) 110:411
27. Jagannath S, Vij R, Stewart K et al (2009) Final results of PX-171–003-A0, part 1 of an open-label, single-arm, phase II study of carfilzomib (CFZ) in patients (pts) with relapsed and refractory multiple myeloma (MM). J Clin Oncol 27(15s), ASCO Annual Meeting Abstract 8504
28. http://www.onyx-pharm.com/view.cfm/690/Onyx-Pharmaceuticals-Announces-Positive-Top-Line-Carfilzomib-Data-from-Phase-2b-Study; 2010.
29. Siegel D, Wang L, Orlowski RZ et al (2009) PX-171-004, an ongoing open-label, phase II study of single-agent carfilzomib (CFZ) in patients with relapsed or refractory myeloma (MM); updated results from the bortezomib-treated cohort. Blood (ASH Annual Meeting Abstract) 114:303
30. Wang L, Siegel D, Kaufman JL et al (2009) Updated results of bortezomib-naïve patients in PX-171-004, an ongoing open-label, phase II study of single-agent carfilzomib (CFZ) in patients with relapsed or refractory myeloma (MM). Blood (ASH Annual Meeting Abstract) 114:302
31. Richardson PG, Weller E, Jagannath S et al (2009) Multicenter, phase I, dose-escalation trial of lenalidomide plus bortezomib for relapsed and relapsed/refractory multiple myeloma. J Clin Oncol 27(34):5713–9

32. Richardson PG, Weller E, Lonial S et al (2010) Lenalidomide, bortezomib, and dexamethasone combination therapy in patients with newly diagnosed multiple myeloma. Blood 116(5):679–86
33. Bensinger W, Wang M, Orlowski RZ et al (2010) Dose-escalation study of carfilzomib (CFZ) plus lenalidomide (LEN) plus low-dose dexamethasone (Dex) (CRd) in relapsed/refractory multiple myeloma (R/R MM). J Clin Oncol 28(15s), ASCO Annual Meeting Abstract 8029
34. Muchamuel T, Aujay M, Bennett MK et al (2008) Preclinical pharmacology and in vitro characterization of PR-047, an oral inhibitor of the 20S proteasome. Blood (ASH Annual Meeting Abstract) 112:3671
35. Zhou HJ, Aujay MA, Bennett MK et al (2009) Design and synthesis of an orally bioavailable and selective peptide epoxyketone proteasome inhibitor (PR-047). J Med Chem 52(9):3028–38
36. Muchamuel T, Kapur S, Kirk CJ et al (2009) Dose intensive administration of PR-047, a novel orally bioavailable inhibitor of the 20S proteasome, is well tolerated in experimental animals. Blood (ASH Annual Meeting Abstract) 114:4910
37. Roccaro AM, Sacco A, Aujay M et al (2010) Selective inhibition of chymotrypsin-like activity of the immunoproteasome and constitutive proteasome in Waldenstrom macroglobulinemia. Blood 115(20):4051–60
38. Huang X, Bailey K, Di Liberto M et al (2008) Induction of sustained early G1 arrest by selective inhibition of CDK4 and CDK6 primes myeloma cells for synergistic killing by proteasome inhibitors carfilzomib and PR-047. Blood (ASH Annual Meeting Abstract) 112:3670
39. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W (2003) Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angew Chem Int Ed Engl 42(3):355–7
40. Fenteany G, Schreiber SL (1998) Lactacystin, proteasome function, and cell fate. J Biol Chem 273(15):8545–8
41. Ling T, Potts BC, Macherla VR (2010) Concise formal synthesis of (−)-salinosporamide A (marizomib) using a regio- and stereoselective epoxidation and reductive oxirane ring-opening strategy. J Org Chem 75(11):3882–5
42. Chauhan D, Catley L, Li G et al (2005) A novel orally active proteasome inhibitor induces apoptosis in multiple myeloma cells with mechanisms distinct from Bortezomib. Cancer Cell 8(5):407–19
43. Singh AV, Palladino MA, Lloyd GK, Potts BC, Chauhan D, Anderson KC (2010) Pharmacodynamic and efficacy studies of the novel proteasome inhibitor NPI-0052 (marizomib) in a human plasmacytoma xenograft murine model. Br J Haematol 149(4):550–9
44. Ahn KS, Sethi G, Chao TH et al (2007) Salinosporamide A (NPI-0052) potentiates apoptosis, suppresses osteoclastogenesis, and inhibits invasion through down-modulation of NF-kappaB regulated gene products. Blood 110(7):2286–95
45. Chauhan D, Singh A, Brahmandam M et al (2008) Combination of proteasome inhibitors bortezomib and NPI-0052 trigger in vivo synergistic cytotoxicity in multiple myeloma. Blood 111(3):1654–64
46. Roccaro AM, Leleu X, Sacco A et al (2008) Dual targeting of the proteasome regulates survival and homing in Waldenstrom macroglobulinemia. Blood 111(9):4752–63
47. Chauhan D, Singh AV, Ciccarelli B, Richardson PG, Palladino MA, Anderson KC (2010) Combination of novel proteasome inhibitor NPI-0052 and lenalidomide trigger in vitro and in vivo synergistic cytotoxicity in multiple myeloma. Blood 115(4):834–45
48. Huang X, Louie T, Di Liberto M et al (2007) Targeting cdk4/6 in combination therapy overcomes proteasome inhibitor resistance in multiple myeloma through synergistic mitochondria depolarization. Blood (ASH Annual Meeting Abstracts) 110:667
49. Kurzrock R, Hamlin P, Younes A et al (2007) Phase 1 clinical trial of a novel proteasome inhibitor (NPI-0052) in patients with lymphomas and solid tumors. Blood (ASH Annual Meeting Abstract) 110:4504
50. Hamlin PA, Aghajanian C, Hong D et al (2008) First-in-human phase 1 dose escalation study of NPI-0052, a novel proteasome inhibitor, in patients with lymphoma and solid tumor. Blood (ASH Annual Meeting Abstracts) 112:4939

51. Hamlin PA, Aghajanian C, Younes A et al (2009) First-in-human phase I study of the novel structure proteasome inhibitor NPI-0052. J Clin Oncol 27(15s):3516, ASCO Annual Meeting Abstract
52. Price T, Padrik P, Townsend A et al (2008) Clinical trial of NPI-0052 (2nd generation proteasome inhibitor) in patients having advanced malignancies with expanded RP2D cohorts in lymphoma and CLL. Blood 112:4934, ASH Annual Meeting Abstract
53. Townsend AR, Millward M, Price T et al (2009) Clinical trial of NPI-0052 in advanced malignancies including lymphoma and leukemia (advanced malignancies arm). J Clin Oncol 27(15s):3582, ASCO Annual Meeting Abstract
54. Spencer A, Millward M, Mainwaring P et al (2009) Phase 1 clinical trial of the novel structure proteasome inhibitor NPI-0052. Blood 114:2693, ASH Annual Meeting Abstract
55. Richardson P, Hofmeister C, Jakubowiak A et al (2009) Phase 1 clinical trial of the novel structure proteasome inhibitor NPI-0052 in patients with relapsed and relapsed/refractory multiple myeloma (MM). Blood (ASH Annual Meeting Abstract) 114:431
56. Dorsey BD, Iqbal M, Chatterjee S et al (2008) Discovery of a potent, selective, and orally active proteasome inhibitor for the treatment of cancer. J Med Chem 51(4):1068–72
57. Piva R, Ruggeri B, Williams M et al (2008) CEP-18770: A novel, orally active proteasome inhibitor with a tumor-selective pharmacologic profile competitive with bortezomib. Blood 111(5):2765–75
58. Trippier PC, McGuigan C, Balzarini J (2010) Phenylboronic-acid-based carbohydrate binders as antiviral therapeutics: monophenylboronic acids. Antivir Chem Chemother 20(6):249–57
59. Sanchez E, Li M, Steinberg JA et al (2010) The proteasome inhibitor CEP-18770 enhances the anti-myeloma activity of bortezomib and melphalan. Br J Haematol 148(4):569–81
60. Marangon E, Sala F, Sessa C, et al. Pharmacokinetics and pharmacodynamics of the new proteasome inhibitor CEP-18770 Preliminary results from a phase I study. J Am Soc Mass Spectrom 2009;20(5, Supplement 1):Annual ASMS Meeting Abstract 452
61. Kupperman E, Lee EC, Cao Y et al (2010) Evaluation of the proteasome inhibitor MLN9708 in preclinical models of human cancer. Cancer Res 70(5):1970–80
62. Donelan J, Bannerman B, Bano K et al (2009) Antitumor activity of MLN9708, a second-generation proteasome inhibitor, in preclinical models of lymphoma. Blood 114:3724, ASH Annual Meeting Abstract
63. Lee E, Bannerman B, Fitzgerald M et al (2009) MLN9708 elicits pharmacodynamic response in the bone marrow compartment and has strong antitumor activity in a preclinical intraosseous model of plasma cell malignancy. Blood 114:1834, ASH Annual Meeting Abstract
64. Fitzgerald M, Cao Y, Bannerman B et al (2009) Evaluating the antitumor activity of MLN9708 in a disseminated mouse model of double transgenic iMyc Ca/Bcl-XL plasma cell malignancy. Blood 114:3835, ASH Annual Meeting Abstract
65. Janz S, Van Ness BG, Neppalli V et al (2009) The novel proteasome inhibitor MLN9708 demonstrates efficacy in a genetically-engineered mouse model of denovo plasma cell malignancy. Blood 114:3849, ASH Annual Meeting Abstract
66. Rodler ET, Infante JR, Siu LL et al (2010) First-in-human, phase I dose-escalation study of investigational drug MLN9708, a second-generation proteasome inhibitor, in advanced nonhematologic malignancies. J Clin Oncol 28:3071, ASCO Annual Meeting Abstract
67. Kloetzel PM, Ossendorp F (2004) Proteasome and peptidase function in MHC-class-I-mediated antigen presentation. Curr Opin Immunol 16(1):76–81
68. Kuhn DJ, Hunsucker SA, Chen Q, Voorhees PM, Orlowski M, Orlowski RZ (2009) Targeted inhibition of the immunoproteasome is a potent strategy against models of multiple myeloma that overcomes resistance to conventional drugs and nonspecific proteasome inhibitors. Blood 113(19):4667–76
69. Muchamuel T, Basler M, Aujay MA et al (2009) A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. Nat Med 15(7):781–7

70. Trikha M, Corringham R, Klein B, Rossi JF (2003) Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. Clin Cancer Res 9(13):4653–65
71. Yasui H, Hideshima T, Richardson PG, Anderson KC (2006) Novel therapeutic strategies targeting growth factor signalling cascades in multiple myeloma. Br J Haematol 132(4): 385–97
72. Hong DS, Angelo LS, Kurzrock R (2007) Interleukin-6 and its receptor in cancer: implications for Translational Therapeutics. Cancer 110(9):1911–28
73. Voorhees PM, Chen Q, Kuhn DJ et al (2007) Inhibition of interleukin-6 signaling with CNTO 328 enhances the activity of bortezomib in preclinical models of multiple myeloma. Clin Cancer Res 13(21):6469–78
74. Voorhees PM, Chen Q, Small GW et al (2009) Targeted inhibition of interleukin-6 with CNTO 328 sensitizes pre-clinical models of multiple myeloma to dexamethasone-mediated cell death. Br J Haematol 145(4):481–90
75. Singh AV, Bandi M, Aujay M et al (2009) PR-924, a selective inhibitor of the immunoproteasome subunit LMP-7 blocks multiple myeloma cell growth both in vitro and In vivo. Blood 114:612, ASH Annual Meeting Abstract
76. Jagannath S, Barlogie B, Berenson JR et al (2005) Bortezomib in recurrent and/or refractory multiple myeloma. Initial clinical experience in patients with impaired renal function. Cancer 103(6):1195–200
77. Chanan-Khan AA, Kaufman JL, Mehta J et al (2007) Activity and safety of bortezomib in multiple myeloma patients with advanced renal failure: a multicenter retrospective study. Blood 109(6):2604–6
78. Blade J, Sonneveld P, SanMiguel F et al (2008) Pegylated liposomal doxorubicin plus bortezomib in relapsed or refractory multiple myeloma: efficacy and safety in patients with renal function impairment. Clin Lymphoma Myeloma 8(6):352–5