Potential for proteasome inhibition in the treatment of cancer

Julian Adams

Proteasome inhibition is a new approach to treating cancer. Proteasome inhibitors specifically induce apoptosis in cancer cells, but most proteasome inhibitors are not suitable for clinical development. Peptide boronates overcome the shortcomings of earlier generation proteasome inhibitors, and bortezomib (VELCADE™; formerly PS-341) is the first peptide boronate to enter clinical trials. Preclinical studies of bortezomib have demonstrated antitumor activity in a variety of tumor types. Phase I trials provided evidence of manageable toxicities and support a twice-weekly dosing regimen now being examined in a Phase III study.

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▼ In addition to removing damaged and unneeded proteins, proteasome-mediated proteolysis is a mechanism for mediating important regulatory proteins within the cell. The proteasome quickly and irreversibly eliminates proteins targeted for degradation, making it pivotal in the modulation of activating and repressing signal transduction pathways, including proliferation and cell death. This fundamental role suggests that the proteasome might provide a novel cellular target for anticancer therapy, and animal studies with several proteasome inhibitors have supported this possibility. Bortezomib (VELCADE™; formerly PS-341, MLN341, and LDP-341) is being developed in oncology and is the first proteasome inhibitor to be tested in clinical trials. This review article provides a summary of the preclinical animal studies with proteasome inhibitors and the clinical experience with bortezomib.

The proteasome and the effects of its inhibition in cells

The 26S proteasome is a large, multi-subunit protein composed of a 20S core proteolytic complex and two 19S regulatory subunits present in the cytoplasm and the nucleus of all eukaryotic cells. Proteins that have been targeted for degradation by the proteasome are covalently modified with a poly-ubiquitin protein chain that is recognized by the 19S subunit (Fig. 1). Once recognized by the regulatory complex, the ubiquitin chain is removed and the protein is denatured and fed into the center of the 20S complex. The proteasome is unique among proteases in that it has threonine residues at each of its catalytic sites [1,2].

Proteolysis by the 26S proteasome is an essential metabolic process. Many investigators have demonstrated the effects of proteasome inhibition on the stability of various cell cycle–regulatory proteins, especially those that are short-lived (Table 1), and bypassing the cell's regulated dictation for their degradation has been implicated in sensitizing cells to apoptosis. Camptothecin-bound cleavable complexes are also substrates for the proteasome [3]; thus, inhibition of proteasome activity stabilizes these complexes, appears to further enhance the antitumor activities of camptothecin treatment, and might be able to overcome resistance to this drug class [4].

Although the mechanisms involved are not absolutely clear, cancer cells and normal cells appear to respond differently to the effects of proteasome inhibition: when treated with a proteasome inhibitor, cell-cycle arrest usually occurs in normal cells, whereas cancer cells are more likely to undergo apoptosis. Proliferation rate does not seem to be important in sensitizing cancer cells because malignant cells with a low proliferation rate are as sensitive to proteasome inhibition as rapidly dividing cancer cells [5,6]. It is possible that when the degradation of cell-cycle regulators is abrogated as a consequence of proteasome inhibition, cellular checkpoint mechanisms block the initiation of mitosis in normal cells, and cell division can resume only after the proteasome's

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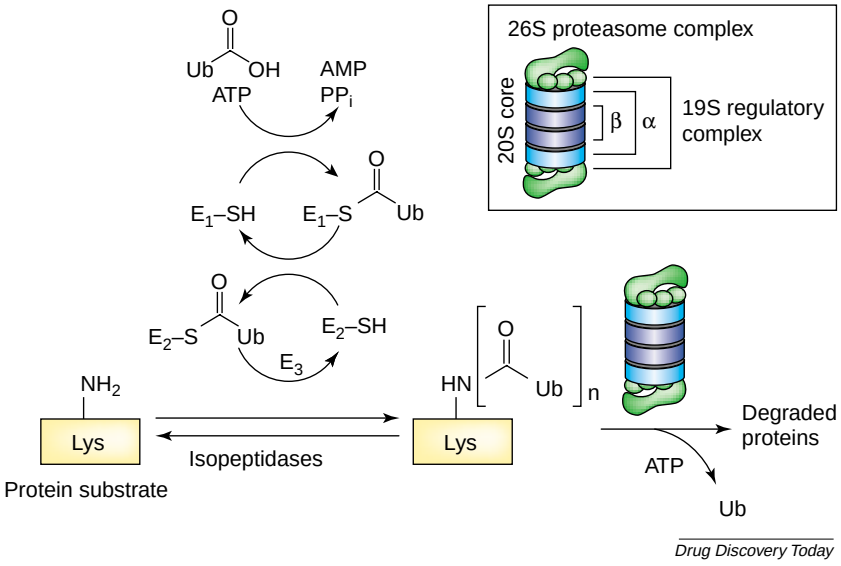

Figure 1. The 26S proteasome structure and the ubiquitin-proteasome pathway. Poly-ubiquitination marks proteins for degradation by the proteasome; tight regulation of this process confers specificity on protein degradation through the ubiquitin-proteasome pathway. Three classes of enzymes are required during ubiquitination. Ubiquitin is activated by attachment to an E1 protein, which transfers ubiquitin to an E2 carrier protein. Degradation signals are recognized by the final enzyme in the pathway, the ubiquitin-protein ligase E3, which catalyzes the final transfer of ubiquitin from the E2 to a lysine on the target protein. The proteasome is a multi-subunit complex (inset) that includes a 20S core and two 19S regulatory caps. The outer α rings of the 20S core restrict access to the six proteolytic sites within the inner β rings; the 19S caps recognize poly-ubiquitinated proteins, remove the ubiquitin chain for recycling and denature the protein so that it can pass through the α rings to the proteolytic chamber. Abbreviations: Ub, ubiquitin; Lys, amino group on side chain of lysine. Copyright 2000, Millennium Pharmaceuticals (http://www.mpi.com).

proteolytic activities have been recovered. By contrast, the genetic modifications that accompany cancer cell transformation are thought to disable cellular checkpoint mechanisms. One hypothesis is that proteasome inhibition could reverse some of the changes that perpetuate proliferation and block apoptosis pathways in cancer cells. For example, the cyclin-dependent kinase inhibitor p27 is degraded by the proteasome [7], and the decreased levels of p27 observed in some malignant cells occur through an increased rate of proteasome-mediated degradation [8,9]. The treatment of cancer cells with proteasome inhibitors results in cell-cycle arrest and a coordinated increase in p27 levels in advance of apoptosis [10,11].

In addition to stabilizing transient regulators of the cell cycle, proteasome inhibition results in the abrogation of nuclear factor-kappaB (NF-κB)–mediated transcription. In quiescent cells, the inhibitor protein, inhibitor-kappaB (IκB), sequesters NF-κB in the cytoplasm, thereby preventing transcriptional activation of NF-κB–regulated genes. Chemotherapy, radiation and other cellular stresses cause the phosphorylation of IκB and its subsequent ubiquitination and degradation by the proteasome, thereby releasing NF-κB [12–14]. The transcription factor then translocates to the nucleus where it binds its consensus sequences within the promoters of genes encoding cytokines [15], cell-adhesion molecules [16] and anti-apoptotic proteins [17,18] (Fig. 2). Thus, the activation of this pathway can stimulate proliferation, prevent apoptosis, and reduce the effectiveness of chemotherapy or radiation therapy [19]. In addition, the NF-κB pathway is activated in many tumor types and the consequences of such activation led investigators to examine the effects of its inhibition in cell culture systems. Duffey *et al.* [20] found that NF-κB activity was required for *in vitro* and *in vivo* survival of a head and neck squamous cell carcinoma (HNSCC) cell line. In addition, chemotherapy-resistant cell lines might also depend on NF-κB activity: a dominant negative IκB super-repressor — a mutant form of IκB that is resistant to proteasome-mediated degradation — caused cell death in melphalan-resistant myeloma cells [21]. Colon cancer cells transfected with an IκB super-repressor were more sensitive to SN-38 (a camptothecin related to CPT-11) than were untransfected cells [22]. These findings suggest that NF-κB activation can be involved in cancer cell survival and chemoresistance, and that NF-κB inhibition can sensitize transformed cells to apoptosis.

Small molecule inhibition of the proteasome

The most specific proteasome inhibitors fall into five classes distinguished by the pharmacophore that interacts with the active site threonine in the proteasome: peptide aldehydes, peptide boronates, peptide vinyl sulfones, peptide epoxyketones and β-lactone inhibitors (reviewed in [1]). The peptide moiety — found in all but the β-lactones — is required for binding to the substrate recognition pocket in the proteasome. Animal data are available only for the peptide aldehydes, peptide boronates and β-lactone inhibitors (Table 2), whereas studies in the other classes have been limited to cell-line experiments. Bortezomib, a peptide boronate, is the first proteasome inhibitor to be studied in humans.

Table 1. Some proteasome substrates

Protein name	Protein function
Cell division	
Topoisomerase II	DNA 'unwinding' [55]
Topoisomerase I	DNA 'unwinding' [3]
Pro- and anti-apoptosis factors	
XIAP	Inhibitor of apoptosis [56]
Bax	Pro-apoptosis factor [57]
Bcl-2	Inhibitor of apoptosis [58]
Survivin	Inhibitor of apoptosis [59]
Securin	Anaphase-promoting factor [60]
Cell-cycle progression	
Cyclin E	Kinase activator [61]
p27 KIP1	Cyclin-dependent kinase inhibitor [7]
p21 CIP1/WAF1	Cyclin-dependent kinase inhibitor [62]
Transcriptional regulation	
Androgen receptor	Nuclear steroid receptor; transcription factor [63]
Fos/Jun	Transcription factor; early immediate gene [64]
Id	Transcription factor inhibitor [65]
IκB-α	Inhibitor of NF-κB [66]
Tumor suppression	
p53	Transcription factor [67]
Rb	Inhibitor of E2F [68]

Peptide aldehydes

This class of inhibitors was the first to be developed and is still used in research. Peptide aldehydes rapidly enter the cell and reversibly inhibit the proteasome; however, the active aldehyde is unstable and quickly oxidized to an inactive acid (reviewed in [1] and [23]). Although many of these compounds are potent proteasome inhibitors, all but CEP1612 and MG132 also have significant activity against other cellular proteases (e.g. serine and cysteine proteases). Nevertheless, several early studies with this class hinted at the possible anticancer activity of proteasome inhibitors. Orlowski *et al.* [24] demonstrated that proteasome inhibition with the aldehyde inhibitor Z-LLF-CHO led to upregulation of the transcription factor c-Myc; this protein is required for entry into S phase and can transform cells. Z-LLF-CHO induced apoptosis in *c-myc*-transformed small cell lung cancer or Burkitt's lymphoma cells, but non-transformed primary or immortalized cells were not as sensitive to Z-LLF-CHO. This result not only showed that proteasome inhibitors might be cytotoxic to cancer cells, but also suggested that transformation could sensitize cells to proteasome inhibitor–induced apoptosis, raising the possibility that tumors could be selectively targeted by this class of compounds. The authors did test inhibition of tumor growth in mice bearing Burkitt's lymphoma tumors: a single injection of Z-LLF-CHO delayed tumor growth with no adverse effects. Although the antitumor effect was modest, the authors noted that treatment with this compound was limited by its solubility; however, further *in vivo* studies with Z-LLF-CHO do not appear to have been conducted.

Experiments with this class of inhibitors also defined several other properties of proteasome inhibitors: their ability to induce apoptosis even in cells with disabled pro-apoptotic systems or those overexpressing anti-apoptotic factors; selective activity against transformed cells; and combinatorial effects with chemotherapy or radiation. For example, Bcl-2 overexpression was unable to protect Jurkat T cells from apoptosis induced by CEP1612, and $p53^{-}$ PC-3 prostate or MDA-MB-231 breast cancer cells were also sensitive to CEP1612 [11]. In this study, An *et al.* also showed that CEP1612 did not appear to induce apoptosis in a normal human fibroblast cell line (WI38); by contrast, transformation of WI38 with SV40 sensitized these cells to CEP1612-mediated apoptosis. Several studies with MG132 demonstrated that proteasome inhibition could sensitize cells to radiation or chemotherapy. HG-My-Z Hodgkin's lymphoma cells are moderately radioresistant; however, pretreatment with MG132 increased the sensitivity of these cells to radiation [25]. Although these cells do have some constitutive NF-κB activity, MG132 did not affect NF-κB activity, suggesting that NF-κB might not always be directly involved in the pro-apoptotic effect of MG132 in these cells. Pancreatic cancer cell lines were similarly sensitized to etoposide (VP16) or doxorubicin [26].

Desai *et al.* [3] also uncovered a possible mechanism for enhanced activity of camptothecins when combined with a proteasome inhibitor. Topoisomerase I relieves DNA supercoiling by introducing a nick in one DNA strand. The cleavable complex is an intermediate in this reaction in which topoisomerase I is covalently bound to one DNA strand; stabilization of the cleavable complex by camptothecins eventually leads to irreparable double-stranded DNA breaks. One mechanism for reducing camptothecin-mediated DNA damage might involve degradation of camptothecin-stabilized cleavable complexes by the proteasome. In addition, the proteasome is involved in downregulation

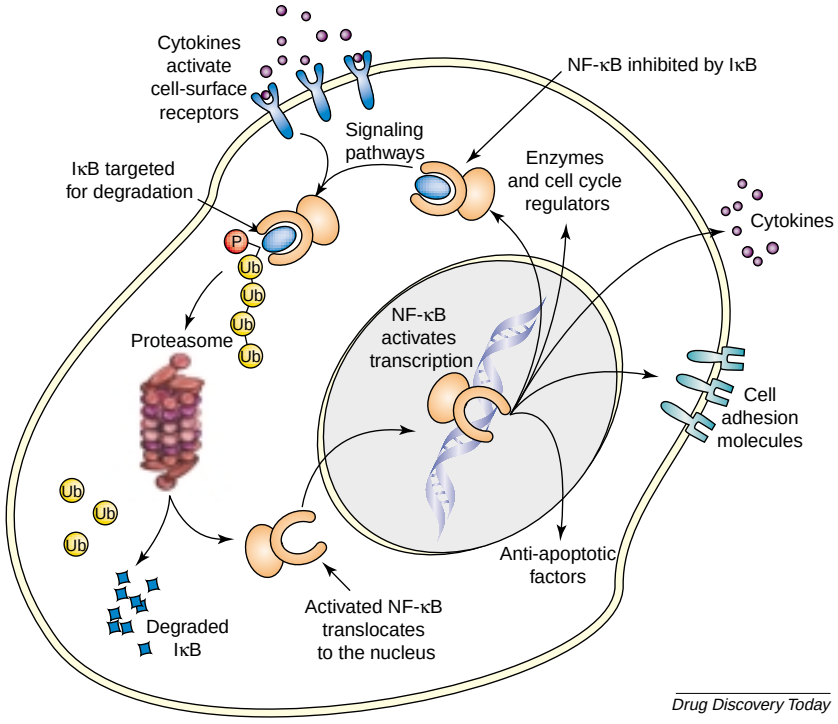

Figure 2. Nuclear factor-kappaB (NF-κB) activation pathway. Normally, NF-κB is found sequestered by IκB in the cytoplasm. Upon stimulation, IκB is marked for degradation by polyubiquitination; the proteasome degrades IκB, releasing NF-κB, which then enters the nucleus. In the nucleus, NF-κB activates multiple cellular responses that can promote cell survival and proliferation. This pathway is also autoregulatory, as NF-κB and IκB expression are under the control of κB promoter elements. Abbreviations: IκB, inhibitor κB; NF-κB, nuclear factor-κB; P, phosphorylated serine; Ub, ubiquitinated lysine. Copyright 2000, Millennium Pharmaceuticals (http://www.mpi.com).

of topoisomerase I after CPT-11 treatment, and cells that efficiently downregulate topoisomerase I are more resistant to camptothecins [27]. Cotreatment of several breast or colon cancer cell lines with MG132 and CPT-11 prevented topoisomerase I downregulation and increased CPT-11 sensitivity [27]. Lauricella *et al.* [28] also observed synergy between camptothecin and MG132 in Y79 retinoblastoma cells.

In vivo studies with peptide aldehydes are limited. In addition to the findings of Orlowski *et al.* with Z-LLF-CHO [24], Golab *et al.* [29] found that PSI – a specific but less potent inhibitor than MG132 – had only moderate activity in the C-26 murine colon cancer model. Cotreatment of C-26 mice with PSI and tumor necrosis factor greatly improved survival, and in some cases, led to complete regression of the tumor. CEP1612 — which appears to be as potent and specific as MG132 [1] — was tested in human lung cancer xenografts [30]. Daily treatment with CEP1612 led to a 68% reduction in tumor growth compared with tumors in untreated mice. Treated mice had no evidence of toxicity (i.e. weight loss, decreased activity or anorexia).

β-lactone inhibitors

Lactacystin is a natural product produced by *Streptomyces* [1]. The cell membrane is impermeable to lactacystin, and lactacystin itself does not react with the proteasome. In cell culture media, however, lactacystin is spontaneously converted to a reactive β-lactone that easily traverses the plasma membrane. The β-lactone inhibits the proteasome through reaction with the hydroxyl group on the active site threonine to form an acyl enzyme conjugate. Under intracellular conditions though, the active β-lactone is a highly unstable molecule that is rapidly inactivated. Despite this drawback, lactacystin is more selective than the peptide aldehydes — having little affinity for cysteine proteases. Chronic lymphocytic leukemia cells (CLL) isolated from patients were tested for sensitivity to radiation and lactacystin; even lines that were resistant to radiation were still sensitive to lactacystin [31]. In addition, only 20% of lymphocytes isolated from non-cancer patients became apoptotic at lactacystin concentrations that led to greater than 90% apoptosis in CLL cells. The proteasome also affects topoisomerase II levels, and the relevance of this finding was demonstrated by combination studies of lactacystin with the topoisomerase II inhibitor etoposide [4]. When colon or ovarian cancer cells were grown under glucose starvation or hypoxic conditions, topoisomerase II levels decreased, leading to decreased effectiveness of etoposide. Combined treatment with lactacystin prevented topoisomerase II downregulation and restored etoposide sensitivity. These conditions might model those found in tumors and suggest that proteasome inhibitors might be active under conditions that protect cells from other chemotherapeutics.

Although these studies demonstrate some of the potential of proteasome inhibitors, lactacystin is not being developed in oncology. However, MLN519 (formerly PS519 and LDP519) is a β-lactone related to the active metabolite of lactacystin that is being investigated for the prevention of reperfusion injury [32–34].

Table 2. Animal studies with proteasome inhibitors

Proteasome inhibitor	Tumor model	Summary of findings
Peptide aldehydes		
Z-LLF-CHO	Human Burkitt's lymphoma	Antitumor activity seen with Z-LLF-CHO but limited by the compound's solubility [24]
PSI + TNF	Murine C-26 carcinoma	PSI alone or TNF had moderate antitumor activity, but combination increased survival and led to a cure rate of 50% [29]
CEP1612	Human A-549 lung adenocarcinoma	CEP1612 reduced tumor growth as a single agent with no evidence of toxicity [30]
β-lactone inhibitor		
Lactacystin + etoposide	Human HT-29 colon cancer	At low doses of lactacystin, the combination of lactacystin and etoposide resulted in substantial inhibition of tumor growth without toxicity. Lactacystin might have prevented topoisomerase II downregulation under hypoxic or glucose starvation conditions [4]
Peptide boronates		
Bortezomib	Human p53^{-} PC-3 prostate cancer	Drug distribution in tissues was examined, and bortezomib levels were found to be very low in testes, brain and skin; single-agent treatment led to tumor regression [35]
Bortezomib + radiation, cyclophosphamide, 5-FU, paclitaxel, or doxorubicin	Murine Lewis lung carcinoma and human EMT-6 breast cancer	Bortezomib was effective as a single agent and enhanced cyclophosphamide, radiation and cisplatin action. Bortezomib treatment reduced metastatic disease in the Lewis lung carcinoma model [36]
Bortezomib + SN-38	Human LoVo colon cancer	NF-κB activation follows SN-38 treatment and protects LoVo cells from apoptosis. Adding bortezomib prevented NF-κB activation and enhanced SN-38 cytotoxicity [10]
Bortezomib + gemcitabine	Human MIA-PaCa-2 pancreatic cancer	Bortezomib induced apoptosis in Bcl-2-overexpressing cells and was not dependent on p53 activity [69]
Bortezomib + radiation	Murine TrampC1 prostate cancer	Bortezomib was at least additive when combined with radiation *in vivo* [38]
Bortezomib	Human mantle cell lymphoma	Bortezomib-treated mice showed little or no gross evidence of mantle cell lymphoma (MCL) tumor involvement; untreated mice showed MCL in the peritoneum, mesentery and parenchymatous organs [70]
Bortezomib + radiation	Human LoVo colon cancer	Bortezomib pretreatment enhanced radiation-induced apoptosis with no evidence of toxicity [39]
Bortezomib + CPT-11	Human BxPc-3 pancreatic cancer	Bortezomib was effective either as a single agent or in combination with CPT-11, with no evidence of toxicity. Complete tumor regression was seen in some mice treated with the combination [71]
Bortezomib	Murine PAM-212 and human UM-SCC-11B squamous cell carcinoma	Bortezomib treatment reduced tumor growth and in some cases led to tumor regression in xenografts [37]
Bortezomib	Human RPMI8226 multiple myeloma	Myeloma xenografts were resistant to dexamethasone, but bortezomib-treated mice showed tumor growth inhibition and prolongation of median survival compared with control animals [44]
Bortezomib + daclizumab	Human MET-1 adult T-cell leukemia	Bortezomib alone did not prolong survival of mice with these aggressive tumors, but the combination of daclizumab (humanized anti-9IL-2Rα antibody) improved survival and induced complete remissions in some animals [72]

Peptide boronates

The shortcomings of the peptide aldehyde inhibitors led Adams *et al.* [23] to synthesize other compounds that might be more effective proteasome inhibitors. Boronic acid derivatives of tripeptide aldehydes were the most potent compounds tested. In fact, this class was considerably better than the aldehyde parent molecules: MG262, a tripeptidyl boronic acid, was greater than 100 times more potent than the equivalent tripeptidyl aldehyde MG132 (K_i = 0.03 versus 4.0 nM). A further improvement in specificity was gained by truncation of the tripeptide to a dipeptide. One of these dipeptides, bortezomib, was still approximately six times more potent (K_i = 0.62 nM) than MG132 and offered advantages in synthesis, stability and specificity for the proteasome over thiol and serine proteases. Thus, dipeptidyl boronic acids are potent, highly specific inhibitors of the proteasome.

Bortezomib has been the best-studied boronic acid inhibitor and is the only proteasome inhibitor to be tested in human patients. Several initial studies revealed the potential for bortezomib as an anticancer agent. Bortezomib had a pattern of cytotoxicity that was unlike other compounds in the National Cancer Institute (NCI) database and was effective against a broad range of tumor types in the NCI 60-cell line screen [35]. Adams *et al.* [35] and Teicher *et al.* [36] also showed this compound was active against prostate and breast cancers in *in vivo* mouse models. Further studies with bortezomib demonstrated that this compound was active as a single agent and, in combination with standard anticancer treatments in preclinical studies, exhibited preferential activity against transformed cells over normal cells and was active under conditions that otherwise render standard classes of chemotherapies ineffective.

Single agent and combination therapy experiments have been conducted in several solid tumor and hematologic malignancies, demonstrating the potential for broad applicability of this drug in cancer treatment. Bortezomib led to regression of HNSCC xenografts; in the same study, the authors found p21 induction after bortezomib treatment, and decreased microvessel density in tumors from treated mice [37]. In a mouse model for lung metastases, bortezomib also suppressed the development of metastases both as a single agent and in combination with various chemotherapies [36]. As expected from earlier studies, bortezomib treatment augmented camptothecin and radiation treatments in *in vivo* studies. Low doses of bortezomib were sufficient to delay prostate tumor growth in mouse xenografts when combined with radiation, indicating the radiosensitizing effect of bortezomib [38]. Colon cancer xenografts also responded to bortezomib treatment, especially when combined with radiation therapy [39] or camptothecins [10,40]. In the cell type used by Cusack *et al.*, bortezomib prevented the activation of NF-κB after SN38 treatment, and increased the expression of p21, p27 and p53 [10], thus suggesting several mechanisms for the increased activity of the combination of bortezomib and SN38.

Bortezomib might also be effective under conditions in which other chemotherapeutics fail. Although p53 is a trigger for inducing apoptosis, several studies have shown that bortezomib can induce apoptosis even in cell lines that do not express functional p53 [35,41]. The mechanisms that protect cells from specific drugs or a broad range of agents do not appear to be protective against bortezomib-induced apoptosis. Myeloma cells that were resistant to common antimyeloma therapies were as susceptible to bortezomib-induced apoptosis as drug-sensitive cell lines [42]. In addition, Steiner *et al.* [43] expressed multidrug-resistance proteins in myeloma cells and then assayed these cells for apoptosis after bortezomib treatment. Expression of MRP3 or MRP5 was not sufficient for protection from apoptosis. These results suggest that bortezomib might be effective even in cancers that are refractory to chemotherapy, that typical drug resistance mechanisms do not necessarily confer resistance to bortezomib, and that bortezomib is not a substrate for the multidrug resistance transporter proteins *in vitro*.

As shown in experiments with lactacystin and CEP1612 [6,11,31], bortezomib was also selectively more toxic to transformed cells than normal cells *in vitro*. Peripheral blood mononuclear cells were ten times more resistant to bortezomib-induced apoptosis than myeloma cells [42]. In a parallel experiment, cultured bone marrow stromal cells (BMSCs) were also resistant to apoptosis after bortezomib treatment. However, binding of myeloma cells to BMSCs induces interleukin 6 secretion from these cells, and bortezomib blocked this response. LeBlanc *et al.* [44] also found that proteasome inhibition was greater in several organs than in the tumors of bortezomib-treated mice, yet these tumors were responsive to bortezomib treatment. Although histological examination of the liver, kidneys, heart, lungs and peripheral blood revealed no indications of toxicity to these tissues, several mice (14%) had to be culled during the experiment, and weight loss was evident in 21% of the mice. Lower doses of bortezomib were much better tolerated, and also improved survival and reduced tumor growth.

Preliminary clinical experience with bortezomib

In 1998, bortezomib became the first proteasome inhibitor tested in human clinical trials with the initiation of three

Table 3. Early Millennium Pharmaceuticals[a]-sponsored Phase I bortezomib trials

Testing center (study number)	Treatment schedule	Cycle length (days)	Dose-limiting toxicities (mg m^{-2})	Maximum tolerated dose (mg m^{-2})
MDACC (19–194)	1× per wk × 4	35	2.0[b]	1.8
MSKCC (98–104)	2× per wk × 2	21	1.56[b]	1.3
UNC/MSKCC (9834 and 0031)	2× per wk × 4	42	1.38[b]	1.04

[a] http://www.mpi.com
[b] Enrollment suspended because of dose-limiting toxicities; maximum dose reached

Phase I dose-escalation trials at four cancer centers in the United States. These three studies tested various dosing regimens (Table 3) in patients with hematologic or solid tumors who were refractory to their current therapy or had no other standard therapy options available. Results from two of these studies have recently been published [45,46], and the full-length report of the third is forthcoming (Papandreou, C.N. *et al.*, unpublished).

Approximately 200 patients have now been treated in Phase I clinical studies (including the NCI-Cancer Treatment Evaluation Program (CTEP)-sponsored studies), and bortezomib has been well tolerated. Some patients experienced low-grade fever and/or fatigue after several cycles of bortezomib at doses above 1.0 mg/m^2. Thrombocytopenia was also observed, but this toxicity was not considered dose limiting and did not require supportive transfusions in any patient. As seen in preclinical animal studies, some patients developed low-grade diarrhea that could be prevented with prophylactic loperamide treatment. In addition, several patients experienced peripheral neuropathy (PN). However, many of these patients were previously treated with platinum- or taxane-containing regimens. Participants in ongoing trials are being monitored to ascertain whether patients treated with prior neurotoxic chemotherapy, or having pre-existing PN, are more likely to experience PN from bortezomib treatment; early indications suggest that dose or schedule modification at the first signs of PN can avert more serious symptoms.

Recent results of two bortezomib combination trials were presented at the most recent annual meeting of the American Society of Clinical Oncology (ASCO). These studies examined bortezomib and gemcitabine [47], and bortezomib and irinotecan [48], in the treatment of resistant and refractory solid tumors. Results suggested that the addition of bortezomib to gemcitabine or irinotecan did not generate additional toxicities; common adverse events were fatigue, diarrhea and thrombocytopenia. In addition to these studies, bortezomib is currently being evaluated in combination with radiation, docetaxel, doxorubicin, paclitaxel, carboplatin, oxaliplatin, gemcitabine/carboplatin or 5-fluorouracil/leucovorin. At present, more than 20 Phase I and II studies are ongoing at NCI-CTEP cancer centers throughout North America. Additional information regarding the NCI's clinical investigation of bortezomib is available on the NCI's website (http://www.cancer.gov/search/clinical_trials).

The preclinical data, combined with preliminary evidence of activity and acceptable toxicity in Phase I trials, provide convincing grounds for further clinical development of the drug. On the once-weekly treatment regimen, several patients with androgen-independent prostate cancer experienced a decrease in prostate specific antigen and serum interleukin 6 levels [49,50]. Additionally, one patient with non-small cell lung cancer experienced a partial response (>50% reduction in tumor mass) on twice-weekly bortezomib at a dose of 1.56 mg m^{-2} [45]. At a dose that resulted in 68% proteasome inhibition, lung metastases in two melanoma patients also responded to a lower dose of bortezomib given on the twice-weekly treatment regimen [51].

Considerable activity has also been evident in resistant and refractory multiple myeloma, with patients showing significant reductions in myeloma-related immunoglobulins and marrow plasmacytosis [52,53]. Preliminary results from the first 70 relapsed and refractory multiple myeloma patients treated in a Phase II trial showed a greater than 90% decline in paraprotein in 20% of these patients [52]. In addition, 39% of patients showed a greater than 50% decline in paraprotein, and complete remission was reported in some patients. Final data for the entire patient population (n = 202) showed an overall response rate (complete, partial or minor responses) of 35% [54]. Complete responses in this trial required the absence of paraprotein by immunofixation, less than 5% plasma cells in the bone marrow, no new bone disease and no plasmacytomas, and were confirmed by an independent review committee. With these strict criteria, 4% of patients in this trial attained a complete response; an additional 6% met all the above criteria except for the absence of paraprotein by immunofixation. These promising results have led to the initiation of an international Phase III trial of bortezomib versus high-dose dexamethasone in relapsed and refractory multiple myeloma.

Conclusion

Proteasome inhibitors exhibit unique characteristics compared with other antineoplastics: activity in non-dividing cells, specificity for transformed cells over normal cells, the ability to induce apoptosis in cells with disabled pro-apoptotic systems, bypass of drug resistance pathways and additive or synergistic activity with chemotherapy or radiation. Animal tumor models were used to validate the effectiveness of these compounds as anticancer agents, but peptide aldehydes, β-lactones and other classes have not proven suitable for *in vivo* or clinical use. Peptide boronates lack the shortcomings of earlier-generation proteasome inhibitors, and bortezomib is the first peptide boronate—and the first proteasome inhibitor—to be tested in humans. Phase I studies showed that this compound is well tolerated as a single agent and provided the rationale for phase II and phase III trials in multiple myeloma. Additional trials of bortezomib, in combination with other cytotoxic regimens, will focus on its activity in solid tumors.

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