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Bortezomib is a novel small molecule that has antitumor activity in preclinical studies and in patients with myeloma.

Bortezomib (PS-341): A Novel, First-in-Class Proteasome Inhibitor for the Treatment of Multiple Myeloma and Other Cancers

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Background: Multiple myeloma (MM) is an incurable malignancy that is diagnosed in approximately 15,000 people in the United States each year. The novel proteasome inhibitor bortezomib has shown antitumor activity in preclinical studies and has entered clinical trials, with encouraging results to date.

Methods: We review and summarize preclinical work demonstrating the tumoricidal effects of proteasome inhibition, focusing on the potent and selective proteasome inhibitor bortezomib, the first such agent to progress to clinical trials. We also address the potential for bortezomib as a therapy for MM.

Results: In preclinical studies bortezomib appears not only to have activity against MM cells, but also to downregulate protective interactions with bone marrow stromal cells and to inhibit blood vessel development. Proteasome inhibition also has been shown to interfere with protective interactions between MM cells and the bone marrow, and to restrict tumor-associated angiogenesis in preclinical models.

Conclusions: Proteasome inhibition is a promising new investigational avenue for cancer therapy. Bortezomib is currently available for the treatment of relapsed and refractory MM. Further trials are underway to assess the safety and efficacy of this agent in MM and a range of other cancers.

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Introduction

The maintenance of cellular homeostasis and the ability of cells to respond to their environment depend on the orderly degradation of key regulatory proteins and their inhibitors. The 26S proteasome plays an essential role in the targeted degradation of such proteins and is therefore involved in the activation and inactivation of many cellular processes. Indeed, studies using proteasome inhibitors have shown that the proteasome is responsible for the elimination of more than 80% of all cellular proteins.¹ Specific targets of the pro-

teasome include cell-cycle proteins, tumor suppressors, and transcription factors, as well as mutant and damaged proteins.¹

The proteasome has been identified as an excellent target for cancer therapy because of its critical metabolic function. Importantly, preclinical research has shown that cancer cells seem to be more sensitive to the proapoptotic effects of proteasome inhibition than are normal cells.^{2,5} It has also been shown that proteasome inhibition enhances the sensitivity of cancer cells to traditional anticancer agents in both *in vitro*⁶⁻¹⁰ and *in vivo*^{7,9} preclinical studies.

This review summarizes preclinical work demonstrating the tumoricidal effects of proteasome inhibition, focusing on the potent and selective proteasome inhibitor bortezomib (formerly PS-341, LDP-341, MLN341), the first such agent to progress to clinical trials. In particular, we address the potential for bortezomib as a therapy for multiple myeloma (MM), an incurable malignancy that is diagnosed in approximately 15,000 people in the United States each year.¹¹ A phase I study of bortezomib in hematologic malignancies established a dose schedule for additional clinical studies with a number of responses (including one complete remission) in patients with MM.¹² This, combined with preclinical data of bortezomib activity in MM cell lines, drug-resistant cell lines, and patient-derived tumor lines,¹⁰ prompted the initiation of two phase II trials of bortezomib in patients with MM, both of which are now complete. Results from one of these trials are promising and show that heavily treated relapsed and refractory patients from the first cohort in this trial experienced manageable toxicities with encouraging response rates.¹³ A phase III trial is now underway in patients with relapsed MM, and bortezomib is also currently in single-agent and combination phase I studies in advanced solid tumors.

Protein Degradation by the Proteasome

Protein degradation by the proteasome is a highly selective process that has been described as a specialized case of protein localization.¹⁴ Generally, proteins are directed to the proteasome by the attachment of ubiquitin, a small protein marker that can be recognized by the proteolytic complex. In an initial enzyme-catalyzed reaction, a single ubiquitin molecule is attached to a lysine side chain on the protein substrate. Further ubiquitin molecules are subsequently added to create a polyubiquitin chain.¹⁴

The enzymes involved in ubiquitination are largely responsible for the specificity of protein targeting. Ubiquitin protein ligases recognize degradation motifs on proteins targeted for degradation; in some cases, post-translational modifications (eg, phosphorylation) are necessary for recognition by a ubiquitin protein ligase.¹⁵

The 26S proteasome is a 2.5-MDa multiprotein complex comprised of a 20S core particle and two 19S regulatory particles (one on each end of the core).¹⁶ The 19S regulatory particles contain subunits responsible for binding the polyubiquitin chain and cleaving it from the protein substrate. They also include 6 ATPases, which are thought to be involved in denaturing the target protein and may deliver the substrate into the proteolytic chamber in the 20S subunit.¹

The 20S core particle is a cylindrical complex made up of four stacked rings. The two outer rings bind to the 19S regulatory particles, and the two inner rings each contain three active sites.¹ These active sites account for the three major proteolytic activities of the proteasome, which have been described as chymotrypsin-like, trypsin-like, and post-glutamyl peptide hydrolytic (PGPH).¹⁶ Proteins entering the core particle are

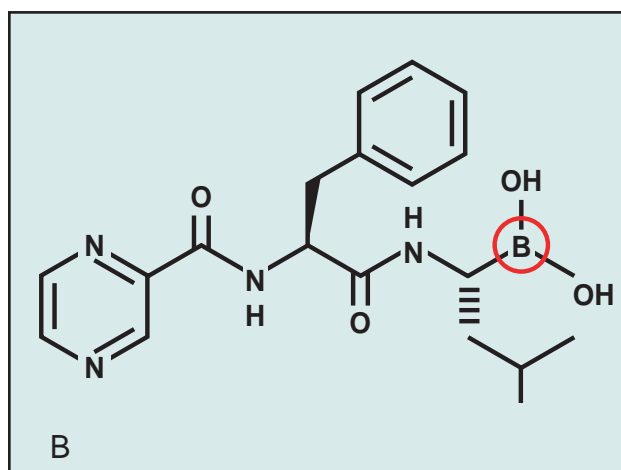
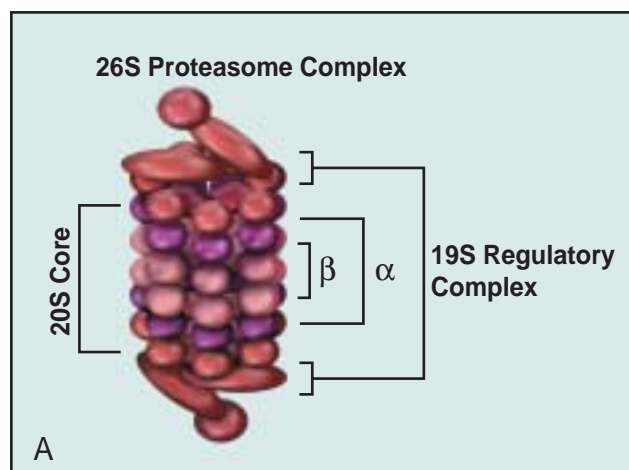


Fig 1. — The 26S proteasome and the proteasome inhibitor bortezomib (PS-341). (A) The proteasome is a multiprotein complex comprised of a cylindrical 20S core particle associated with two 19S regulatory units. (B) Bortezomib is a dipeptidyl boronic acid that potently and selectively inhibits the activity of the proteasome. Copyright Millennium Pharmaceuticals Inc, Cambridge, Mass.

degraded in a progressive manner, yielding peptides between 3 and 25 amino acids in length that are in turn hydrolyzed by other cellular peptidases¹⁵ (Fig 1).

Proteasome Inhibitors

A variety of compounds, both natural and synthetic, have been found to inhibit protein degradation by the proteasome. Synthetic inhibitors of the proteasome include peptide aldehydes such as Z-Leu-Leu-al (MG132), Z-Ile-Glu(OBut)-Ala-Leu-al (PSI), Ac-Leu-Leu-Nle-al (ALLN), and peptide vinyl sulfones. Natural proteasome inhibitors include lactacystin, epoxyketones such as epoxomicin and eponemycin, and the TMC-95 cyclic peptides.¹⁷ All of these compounds bind to and directly inhibit active sites within the 20S core particle. However, most primarily interfere with the chymotrypsin-like activity of the core particle (the rate-limiting step in proteolysis) and appear to have little effect on the other proteolytic activities.¹⁷ Many of these inhibitors also lack specificity for the proteasome or exhibit unfavorable intracellular kinetics, which make them less suitable for clinical use. For instance, peptide aldehyde inhibitors dissociate rapidly from the proteasome, are inactivated by oxidization, and are removed from the cell by the multidrug transporter system, which allows only short-lived proteasome inhibition.¹⁷ Furthermore, these compounds are known inhibitors of serine and cysteine proteases, including calpains and cathepsins, and thus would not be safe for use in patients.¹⁷ Although the peptide vinyl sulfones and natural inhibitors of the proteasome display less nonspecific activity than the peptide aldehydes, all bind to the 20S core particle in an irreversible manner, such that proteolytic activity cannot be restored upon their removal.¹⁷ Given the fundamental metabolic role of the proteasome, this permanent inhibition would likely be detrimental.

These problems were overcome, however, by replacing the aldehyde group of the synthetic peptide inhibitors with boronic acid. The peptide boronates differ from their aldehyde analogs in that they dissociate more slowly from the proteasome, conferring stable inhibition. Further, the weak interaction between boron and sulphur means that the peptide boronates do not inhibit thiol proteases. The peptide boronic acids are also up to 100-fold more potent than their peptide aldehyde analogs.^{17,18} Dipeptide boronic acid bortezomib is of particular interest from a clinical perspective. This small, water-soluble compound is a potent and selective proteasome inhibitor, which offers the additional advantages of low molecular weight and ease of synthesis.¹⁸ Bortezomib is the first molecule in this class to reach clinical trials in cancer patients.

Multiple Myeloma

Multiple myeloma is a hematologic malignancy typically characterized by the accumulation of clonal plasma cells at multiple sites in the bone marrow. Although the majority of patients respond to initial treatment with chemotherapy and radiation, most eventually relapse due to the proliferation of resistant tumor cells; despite the advent of high-dose chemotherapy with stem-cell transplantation, MM remains incurable.¹⁹ This cytotoxic resistance reflects both the inherent characteristics of the MM cell and the protective interactions between the tumor and the bone marrow microenvironment.¹⁹ This review draws information regarding proteasome inhibitor activity from a range of studies but focuses on the potential ability of bortezomib to overcome mechanisms of resistance in MM.

Activity of Proteasome Inhibitors in MM and Other Cancer Cells

Proteolysis by the 26S proteasome is an essential metabolic process, and inhibition of the proteasome results in growth arrest and cell death.¹⁷ There is strong evidence that the cell death induced by proteasome inhibition is apoptotic. For example, the cell death observed in MM cells exposed to bortezomib in vitro involved caspase-3 activation and annexin V binding.²⁰ Further, gastric cancer cells treated with MG-132 exhibited signs of apoptosis, such as cytoplasmic and nuclear shrinkage, chromatin condensation and fragmentation, DNA laddering, upregulation of the proapoptotic protein Bax, release of mitochondrial cytochrome c, and caspase activation.²¹

In preclinical studies, proteasome inhibition appears to be capable of inducing apoptosis despite the expression of antiapoptotic proteins. Thus, the forced expression of Bcl-2 in MM cells delayed but could not prevent bortezomib-induced cell death.²⁰ Further, bortezomib induced apoptosis in MM cells despite the upregulation of p21 and p27 and irrespective of p53 status.¹⁰

Sensitivity of MM and Other Tumors to Proteasome Inhibition

In laboratory studies, MM cell lines were significantly more sensitive to the proapoptotic effects of bortezomib proteasome inhibition than were bone marrow cells or peripheral blood mononuclear cells from healthy individuals.^{5,6} Similarly, other proteasome inhibitors induced apoptosis in chronic lymphocytic leukemia cells and oral squamous cell carcinoma cells at doses that had no effect on normal human lympho-

cytes or oral epithelial cells, respectively.²⁴ Transformed fibroblasts were also up to 40 times more susceptible to the proapoptotic effects of proteasome inhibition than were primary rodent fibroblasts or immortalized nontransformed human lymphoblasts.⁵

It has also been noted in preclinical studies that actively dividing cells are more sensitive to proteasome inhibition than are quiescent or differentiated cells. For instance, quiescent bovine and human endothelial cells were considerably less susceptible to proteasome inhibition-induced apoptosis using PSI than were proliferating cells.²² While active division does appear to increase sensitivity to proteasome inhibition, it is likely that other mechanisms contribute to the anticancer activity of proteasome inhibitors.

Intracellular Signaling Pathways Affected by Proteasome Inhibition

Inhibition of proteasomal activity results in the accumulation of numerous regulatory proteins within the cell. Proteins stabilized by proteasome inhibition include the tumor-suppressor protein p53 and the cell-cycle proteins p21 and p27.¹⁷ The precise molecular events leading to proteasome inhibitor-induced apoptosis are not yet known. It is possible that proteasome inhibition triggers the apoptotic cascade, in part, by causing the rapid accumulation of incompatible regulatory proteins within the cell.¹⁷

The activation of transcription factor nuclear factor- κ B (NF- κ B) is dependent on proteasome activity. NF- κ B

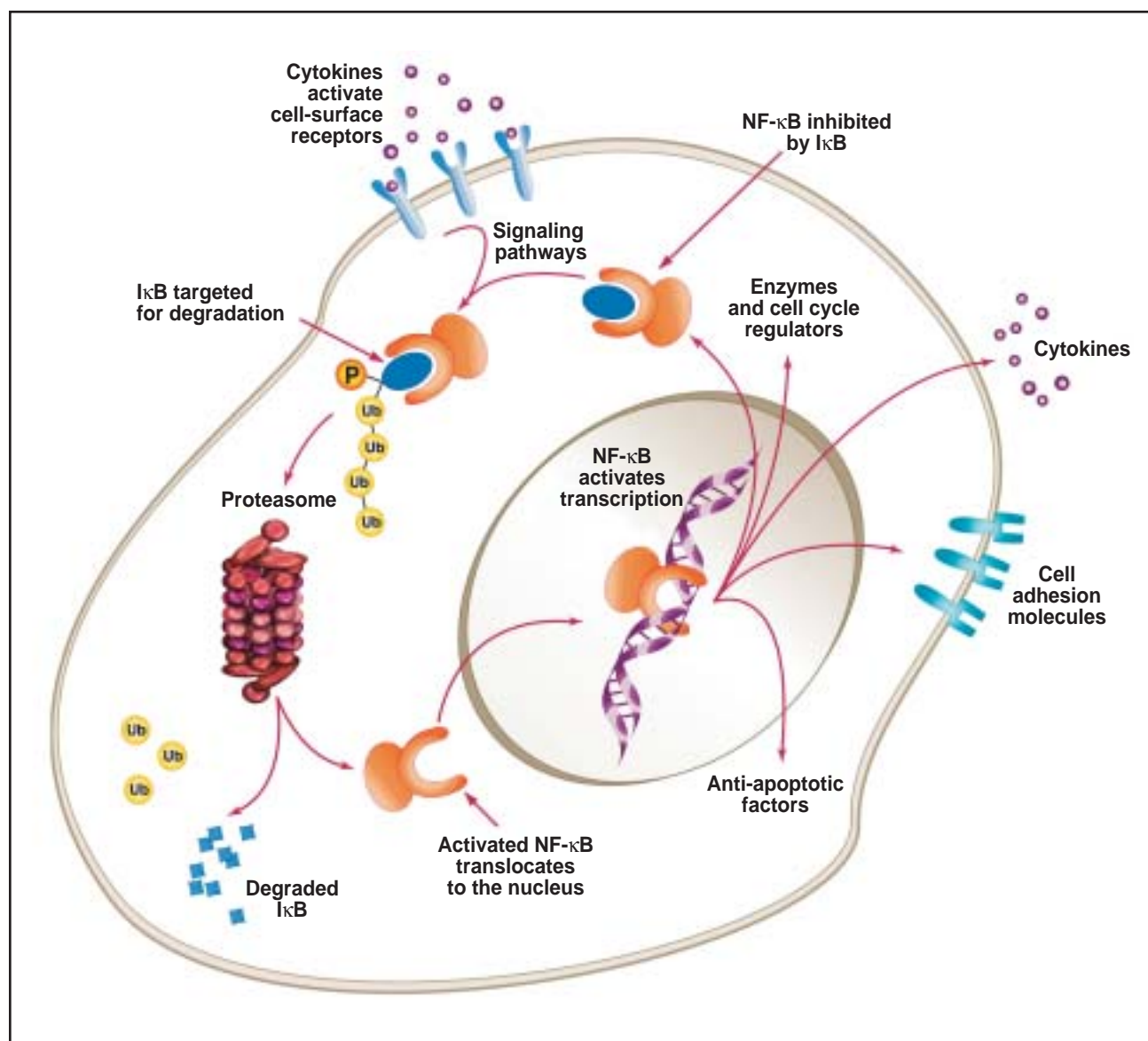


Fig 2. — The activation of NF- κ B. Cell signaling pathways induced by growth factors, chemotherapy, or radiotherapy result in the phosphorylation and proteasomal degradation of I κ B. The transcription factor NF- κ B is thereby released and promotes the expression of cytokines, cell adhesion molecules, and antiapoptotic proteins. Copyright Millennium Pharmaceuticals Inc, Cambridge, Mass.

is normally sequestered in the cytoplasm and rendered inactive by its inhibitor protein I κ B. However, numerous stimuli, including cell stressors, prompt the phosphorylation and subsequent proteasomal degradation of I κ B, thus allowing the release of NF- κ B. The transcription factor then translocates to the nucleus and initiates the transcription of a wide range of genes, including those involved in cell survival, cell adhesion, and cytokine signaling. In MM cells *in vitro*, for example, NF- κ B has been shown to activate the expression of Bcl-2, A1, X chromosome-linked inhibitor of apoptosis protein (XIAP), cellular inhibitor of apoptosis protein 1 (cIAP-1), cIAP-2, and survivin; it has also been shown to initiate the downregulation of Bax²³ (Fig 2).

The NF- κ B pathway is constitutively active in some cancer cells and is associated with resistance to anticancer therapy. Specifically, in preclinical studies, MM tumor cells and the bone marrow of MM patients show enhanced NF- κ B activity, and chemoresistant MM cells have increased NF- κ B activity compared with chemosensitive lines.^{6,24} However, proteasome inhibitors have been shown to stabilize I κ B and prevent the activation of NF- κ B. Thus, in the ARP-1 human MM cell line, bortezomib increased the level of phosphorylated I κ B protein and inhibited constitutive NF- κ B activity.²⁰ Further, bortezomib inhibited the tumor necrosis factor α (TNF- α)-induced activation of NF- κ B in preclinical studies of MM cells in a dose- and time-dependent fashion.²⁵ This is a significant finding, since TNF- α is present in the bone marrow microenvironment and is known to activate NF- κ B-dependent gene expression and proliferation in MM cells.²³

Proteasome inhibition has also been shown to block chemotherapy- and radiotherapy-induced activation of NF- κ B, resulting in enhanced sensitivity to these tumoricidal agents and increased apoptosis in cancer cells *in vitro*. However, the direct inhibition of I κ B α phosphorylation is insufficient to completely inhibit the proliferation of MM cells, suggesting that bortezomib does not act through NF- κ B blockade alone.²⁵

Preclinical Studies of Bortezomib in MM and Other Cancers

Preliminary studies demonstrate that bortezomib is highly toxic against a broad range of cancer cell lines *in vitro*. Bortezomib is the only proteasome inhibitor to have been evaluated in a wide range of murine xenograft models, and these studies have confirmed the activity of the drug *in vivo*.

As a single agent, bortezomib has been shown to potently inhibit both intracellular proteolysis and cell

proliferation in a standard National Cancer Institute (NCI) screen of 60 cell lines derived from multiple human tumors.²⁶ Importantly, unlike most anticancer agents, bortezomib can also overcome multicellular drug resistance *in vitro*. Preclinical studies of four human ovarian and three human prostate carcinoma cell lines demonstrate that, in contrast to most other known anticancer drugs, and with only one exception, bortezomib induced apoptosis at least as effectively in spheroid cell cultures as in monolayer cultures.²⁷ This suggests that bortezomib may be equally effective against solid tumors and hematological malignancies. Indeed, bortezomib has been shown to potently inhibit the growth of tumors in mice bearing human prostate cancer and squamous cell carcinoma xenografts,^{26,28} as well as grafted murine mammary tumors.²⁹ In fact, bortezomib was sufficient to induce complete regression of prostate cancer xenografts in some animals²⁶ and prevent the development of tumors in human mantle cell lymphoma-xenografted mice.³⁰

As a single agent, bortezomib has also been shown to significantly inhibit the growth of human MM xenografted tumors.³¹ The treatment of dexamethasone-resistant MM-xenografted mice with bortezomib (0.05 to 1.0 mg/kg) resulted in significant tumor growth inhibition after as few as 5 days, even at the lowest doses. Indeed, complete tumor regression was seen in some mice receiving 0.5 or 1.0 mg/kg of bortezomib. Importantly, tumor inhibition was accompanied by prolonged median survival (>40%), and both of these effects occurred in a dose-dependent manner. In the same study, it appeared that, in addition to its direct activity against MM cells, bortezomib also inhibited angiogenesis.³¹

The cytotoxic profile of bortezomib in cell culture has been found to be unique, with little similarity to standard or experimental anticancer agents.²⁶ Given its novel mechanism of action, it would be expected that proteasome inhibition might prove particularly effective when used in combination with conventional tumoricidal agents, and numerous studies in both cell culture and murine models have shown that bortezomib enhanced the sensitivity of cancer cells to these agents.^{7-9,28}

The treatment of MM cells with the combination of bortezomib and dexamethasone inhibited cell proliferation in an additive manner.³² Similarly, high doses of bortezomib induced apoptosis in MIA-PaCa-2 human pancreatic cancer cells in culture, while low doses increased the cytotoxicity of gemcitabine in both cultured cells and pancreatic cancer-xenografted mice.⁸ Bortezomib also improved the response of both human colorectal and pancreatic cancer cells to CPT-11 treatment in culture. Further, combined bortezomib and CPT-11 treatment inhibited tumor growth in mice bear-

ing these cancers to a greater extent than either agent alone. These results are likely to reflect the inhibition of CPT-11-induced NF- κ B activation that is observed in cells pretreated with bortezomib.^{7,9} Moreover, bortezomib alone and in combination with other tumoricidal agents was found to inhibit metastatic disease in a murine Lewis lung model.²⁹

Importantly, in preclinical studies, proteasome inhibition also sensitized cancer cells to ionizing radiation. Combined bortezomib treatment and radiotherapy resulted in significantly reduced tumor growth in mice bearing human colorectal or human prostate tumors^{33,34} or grafted murine mammary tumors.

Indeed, proteasome inhibition was sufficient to overcome the resistance of some cell types to conventional therapies. As a single agent, bortezomib was found to have consistent antitumor activity in both chemosensitive and chemoresistant MM cells, with a 50% inhibitory concentration (IC₅₀) of 10 to 20 ng/mL.⁶ At nontoxic doses, bortezomib also sensitized chemoresistant MM cell lines to the chemotherapeutic drugs melphalan, doxorubicin, and mitoxantrone, all of which became cytotoxic at concentrations 10,000-fold to 100,000-fold lower than usual.^{6,10,24} Importantly, this combined treatment had no significant cytotoxic effects on CD34-selected bone marrow or peripheral blood mononuclear cells from healthy individuals.⁶

Bortezomib also overcame the resistance to apoptosis in MM cells that is conferred by interleukin-6 (IL-6), a major growth and survival factor for these cells.¹⁰ This finding suggests bortezomib-mediated proteasome inhibition acts independently of MM cells' intrinsic mechanism of chemoresistance. The mechanisms by which proteasome inhibition overcomes drug resistance are not fully understood. It has become clear, however, that in addition to targeting cancer cells directly, proteasome inhibitors may overcome drug resistance in vivo by interfering with the protective interaction between cancer cells and the bone marrow.¹⁰ In MM, the adherence of tumor to bone marrow stromal cells (BMSCs) provides protection against apoptosis, promotes tumor cell survival and progression, and confers protection against chemotherapeutic drugs.³⁵ One mechanism that has been found to contribute to drug resistance in MM is the β 1-integrin-mediated adhesion of MM cells to fibronectin.^{19,36} Adhesion to fibronectin protects cells from common chemotherapeutic agents such as doxorubicin and melphalan as well as radiation,¹⁹ and other agents that interfere with cell adhesion or signaling events related to adhesion may therefore prove effective against MM.¹⁹

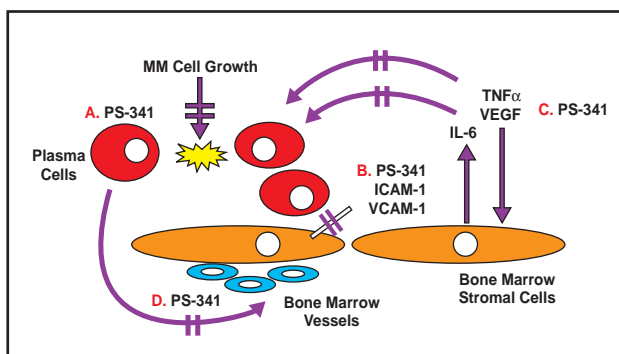


Fig 3. — The activity of bortezomib (PS-341) in MM. In preclinical studies of MM cells, bortezomib acts to (A) directly inhibit the growth of cancer cells, (B) interfere with the adhesion of MM cells to bone marrow stromal cells, (C) inhibit the production of cytokines in the bone marrow, and (D) restrict the development of tumor-associated blood vessels. Modified from Anderson KC. Targeted therapy for multiple myeloma. *Semin Hematol.* 2001;28:288. Reprinted with permission from W. B. Saunders Co.

Of the 52 genes found to be upregulated more than 2-fold in fibronectin-bound MM cells, 11 are known to be NF- κ B responsive.³⁷ Importantly, pretreatment with either MG-132 or bortezomib reversed adhesion-mediated drug resistance and sensitized these cells to cytotoxic agents.³⁷ Further studies of bortezomib activity found that proteasome inhibition decreased the binding of MM cells to BMSCs by 50%.¹⁰ Under normal circumstances, some MM cells produce TNF- α , a cytokine that enhances cell-cell interactions by increasing the secretion of IL-6 from BMSCs and upregulating the expression of the adhesion molecules VLA-4 and LFA-1 on MM cells and the corresponding receptors VCAM-1 and ICAM-1 on BMSCs.^{38,39} Bortezomib prevented the TNF- α -induced, NF- κ B-dependent upregulation of IL-6 and therefore reduced cell adhesion.¹⁰ Moreover, the proliferation of the remaining adherent MM cells was also inhibited by bortezomib (Fig 3).^{10,39}

Proteasome Inhibitors and Angiogenesis

In preclinical studies, proteasome inhibitors have shown greater activity against dividing endothelial cells than against quiescent cells,²² suggesting that they target the aberrant blood vessel development associated with tumor growth. Indeed, lactacystin inhibits the formation of vascular endothelial tubes in vitro and prevents the expression of plasminogen activator, a protease known to be involved in angiogenesis.⁴⁰ Furthermore, lactacystin and the peptide aldehyde inhibitor PSI inhibit blood vessel formation in embryonic chick chorioallantoic membrane (CAM), a densely vascularized tissue commonly used to assess antiangiogenic drugs.^{22,40} Detailed histological analysis shows that proteasome inhibition results in the loss of capillaries and first-order blood vessels in this model.²¹

Evidence that proteasome inhibition has anti-angiogenic activity in murine xenograft models comes from both a study of mice bearing human squamous cell carcinoma tumors and a murine MM study, with mice treated with bortezomib showing reduced tumor blood vessel density and, in the former study, down-regulation of NF- κ B-dependent proangiogenic cytokine expression.^{28,31}

Clinical Studies

To date, bortezomib is the only proteasome inhibitor to have progressed to clinical trials in cancer patients. Bortezomib is cleared rapidly from the plasma compartment, and standard pharmacokinetic parameters have not proven useful in determining the activity of the drug. A novel *ex vivo* pharmacodynamic assay has therefore been used as a guide to dose escalation in phase I trials. This assay efficiently measures residual proteasome activity in blood samples and tumor biopsies, allowing the effects of the drug to be quantified.

Maximum inhibition of 20S activity has been observed 1 hour postdose and, importantly, proteasome inhibition was completely reversible, with a return to baseline by 72 hours postdose.⁴¹ In toxicology studies in rodents and primates, the adverse events associated with bortezomib were primarily gastrointestinal and included anorexia, vomiting, and diarrhea. The inhibition of proteasomal activity by up to 80% was generally well tolerated in these studies.²⁶ Phase I and preliminary phase II data suggest the toxicities associated with bortezomib treatment were manageable and were both dose dependent and reversible. The maximum tolerable dose for bortezomib alone in cancer patients appeared to be influenced by both the tumor type and the treatment schedule. Three dose intensities have been evaluated in phase I trials of advanced solid or hematologic cancers. These schedules administered bortezomib once weekly for 4 weeks (least dose intensive), twice weekly for 2 weeks (moderate dose intensity), and twice weekly for 4 weeks (most dose intensive). The latter regimen, which was assessed in patients with advanced hematologic malignancies,¹² showed a maximum tolerated dose of 1.04 mg/m². The other two regimens, both in patients with advanced solid tumors, showed maximum tolerated doses of 1.8 mg/m² with the least dose-intensive regimen⁴² and 1.56 mg/m² with the regimen of moderate dose intensity.⁴³

Phase I studies of patients with advanced solid tumors have assessed the tolerability of bortezomib administered in combination with either gemcitabine or irinotecan. The phase I study of bortezomib in combi-

nation with gemcitabine is ongoing and aims to establish a maximum tolerated dose in chemotherapy-naïve patients.⁴⁴ Although there was some evidence of biological activity in these patients, particularly in those with pancreatic and lung cancers, preliminary assessments of the activity of bortezomib in combination with other agents for advanced solid tumors await the completion of phase II trials, which are ongoing in lung, prostate, and breast cancers. In a phase I dose-escalation study of 33 patients receiving both bortezomib and irinotecan, toxicities were found to be manageable, and a maximum tolerated dose has not been reached. There was no evidence of additive toxicity in this trial.⁴⁵

Following the phase I observation of the activity of bortezomib in patients with MM,⁴⁶ a phase II study was undertaken in patients with relapsed and refractory disease in the United States.¹³ Patients received 1.3 mg/m² of bortezomib by intravenous push, twice weekly for the first 2 weeks (days 1, 4, 8, and 11) of a 21-day cycle for up to 8 treatment cycles. The addition of dexamethasone was permitted in patients with progressive or stable disease after 2 or 4 cycles, respectively. Response criteria, based on those of Blade and colleagues,⁴⁷ were M protein, soft tissue plasmacytomas, lytic lesions in bone, and percentage of plasma cells in bone marrow. A complete response was defined by a complete absence of M protein with immunofixation confirmation (ie, negative immunofixation), plasma cells in marrow less than 5%, no plasmacytomas, stable skeletal disease, as well as normal serum calcium, where all responses were confirmed 6 weeks after the initial observation.

Of 202 enrolled patients, 193 were evaluable; 92% had been treated with 3 or more of the major classes of drugs commonly used for myeloma, and 91% were refractory to their most recent therapy. The response rate (complete, partial, or minor response) to bortezomib was 35%. Four percent of patients had complete responses, and an additional 6% had 100% reduction in M protein and stable bone disease yet positive immunofixation. The median overall survival was 16 months, with a median duration response of 12 months. Also noted were improved quality-of-life parameters, improved levels of normal immunoglobulins, decreased transfusion requirements, and improvements in hemoglobin levels. While the average patient was heavily pretreated (average prior therapies received = 5), the effects of bortezomib on patients' paraprotein levels appeared to be independent of prior therapies.

No grade 4 adverse events were reported. The most common grade 3 adverse events (>10%) included thrombocytopenia (28%), fatigue (12%), peripheral neuropathy (12%), and neutropenia (11%). In this

study, the drug was associated with manageable toxicities (>10%) of any grade (1-4), including nausea (55%), diarrhea (44%), fatigue (41%), thrombocytopenia (40%), peripheral neuropathy (31%), vomiting (27%), anorexia (25%), pyrexia (22%), anemia (21%), neutropenia (19%), headache (19%), constipation (16%), rash (15%), pain in limb (13%), dizziness (excluding vertigo, 12%), and weakness (11%).

A cumulative dose-related peripheral sensory neuropathy was noted, but there were no cases of grade 4 neuropathy. Although peripheral neuropathy arose or existing neuropathy worsened in 34% of patients, in most cases neuropathy improved during the follow-up period. Of the 33 patients who developed a new neuropathy while on the study, only 1 progressed to grade 3. Of note, 83% of patients had previously received thalidomide, a known neurotoxin. Preliminary results of a recently reported phase II trial in patients with relapsed or refractory myeloma indicate that less-heavily pretreated patients who have earlier-stage disease experienced less toxicity.⁴⁸ A phase III trial of bortezomib in patients with relapsed MM treated with 1 to 3 prior regimens is now underway.

In May of 2003, bortezomib received accelerated FDA approval for the treatment of patients with MM who have received at least two prior therapies and have demonstrated disease progression on the last therapy.

Conclusions

Proteasome inhibition is a promising new investigational avenue for cancer therapy. The proteasome inhibitor bortezomib (previously known as PS-341) is a novel, small molecule that has shown antitumor activity in preclinical studies and has entered clinical trials, with encouraging results to date. Preclinically, bortezomib has exhibited potent activity, enhanced the sensitivity of cancer cells to traditional tumoricidal agents, and appeared to overcome drug resistance. Importantly, in preclinical studies bortezomib appears not only to have activity against MM cells, but also to downregulate protective interactions with BMSCs in the bone marrow microenvironment and to inhibit blood vessel development. Bortezomib is currently available for the treatment of relapsed and refractory MM. Further trials are underway to assess the safety and efficacy of this agent in MM and a range of other cancers.

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