

Bortezomib as a Potential Treatment for Prostate Cancer

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Abstract

Androgen ablation and chemotherapy provide effective palliation for most patients with advanced prostate cancer, but eventually progressing androgen-independent prostate cancer threatens the lives of patients usually within a few years, mandating improvement in therapy. Proteasome inhibition has been proposed as a therapy target for the treatment of solid and hematological malignancies. The proteasome is a ubiquitous enzyme complex that is a hub for the regulation of many intracellular regulatory pathways; because of its essential function, this enzyme has become a new target for cancer treatment. Studies with bortezomib (VELCADE, formerly known as PS-341) and other proteasome inhibitors indicate that cancer cells are especially dependent on the proteasome for survival, and several mechanisms used by prostate cancer cells require proteasome function. Bortezomib has been studied extensively *in vitro* and *in vivo*, and anticancer activity has been seen in cell and animal models for several solid tumor types, including prostate cancer. A Phase I trial to determine the maximum tolerated dose of once-weekly bortezomib has been completed. This trial included a large fraction of patients with androgen-independent prostate cancer. The maximum tolerated dose was reached at 1.6 mg/m². A correlation was seen among bortezomib dose, proteasome inhibition, and positive modulation of serum prostate-specific antigen. There was also evidence of down-regulation of serum interleukin 6, a downstream nuclear factor κ B effector. This Phase I trial and preclinical studies support additional testing of bortezomib in combination with radiation or chemotherapy for androgen-independent prostate cancer.

Introduction

Androgens are necessary for normal prostate cell growth (1), and in animal models, androgen ablation causes cell atrophy and death of prostate epithelial cells (2). Likewise, in the early stages of prostate cancer, the growth of cancerous prostatic epithelial cells is often androgen dependent (3). Androgen ablation remains the main initial treatment of advanced prostate cancer and provides palliation of symptoms and survival benefit. However, androgen-independent cells are eventually selected during androgen ablation therapy (4), and progression to an androgen-independent state remains the primary cause of mortality in these patients within an average of 1.5 years.

Treatment strategies currently available for treating androgen-independent prostate cancer (AIPC; *i.e.*, radiation or chemotherapy) provide temporary palliation, but eventually prostate cancer cells become resistant to chemotherapy and radiation, with ensuing failure to control tumor growth. New treatments and modulation of established treatment regimens (*i.e.*, restoring sensitivity to chemotherapy) are therefore being sought.

Several mechanisms have been proposed to account for the development of androgen independence in prostate cancer cells (4).

Intrinsic activation of receptors or activation of androgen receptor (AR) coregulatory molecules (*e.g.*, ARA55, ARA54, ARA70, BRCA1, and heat-shock proteins) may allow cells to become independent of androgens (intrinsic activation). Induction of growth factors [interleukin 6 (IL-6), insulin-like growth factor-1, and vascular endothelial growth factor/platelet-derived growth factor) may indirectly activate the AR and/or its downstream signaling pathways (5–7). In addition, loss of tumor suppressor activity or proapoptotic factors can contribute to androgen-independent growth (8) and has been reviewed (9). However, it is likely that no single pathway exists for the loss of androgen dependence, and all of these pathways (and possibly others) may work together to bring about androgen independence and disease progression.

In this review we discuss current knowledge about the structure and function of the proteasome and preclinical effects of proteasome inhibition, as well as initial clinical experience with bortezomib (VELCADE), the first proteasome inhibitor, in patients with androgen-independent prostate carcinoma.

The Proteasome: A New Cellular Target for Prostate Cancer Treatment. Bortezomib is a proteasome inhibitor. In preclinical experiments, bortezomib induces growth arrest and apoptosis in many tumor types (10), including androgen-dependent (LNCaP) and androgen-independent prostate cancer cell lines (PC-3 and DU145; Refs. 11–15). As demonstrated in these experiments, proteasome inhibition could be a novel method for treating androgen-dependent and androgen-independent prostate cancer.

The proteasome is a highly conserved and essential mechanism for degrading the majority of intracellular proteins in the eukaryotic cell. Cellular homeostasis and intracellular signaling pathways depend on the proteasome for regulation of key proteins. Encouraging results from Phase I studies of the proteasome inhibitor bortezomib in hematological malignancies (16), AIPC (17, 18), and other solid tumors (19) provide ample justification for additional study of this drug in human malignancies.

The Proteasome and the Ubiquitin-Proteasome Pathway. The proteasome is a large multiprotein complex present in all cells, both in the cytoplasm and nucleus, that degrades ubiquitinated proteins (20, 21). Degradation by the proteasome is a highly regulated process that controls the expression of a wide variety of cellular targets including cyclins (*i.e.*, cyclin B), cyclin-dependent kinase inhibitors (*i.e.*, p21 and p27; Ref. 22), pro- and antiapoptosis factors such as Bax and Bcl-2 (23, 24), early immediate transcription factors such as c-Myc (25), tumor suppressors such as p53 (26), and transcriptional inhibitors such as Id (27) and inhibitor of nuclear factor- κ B (I κ B) α (28). PEST (proline-glutamate-serine-threonine) sequences and/or PEST-like sequences are often involved in the cellular targeting of proteins for degradation (29). Phosphorylation of PEST sequences in response to cellular signals may mediate the initiation of the ubiquitination and the degradative process (30). Phosphorylated PEST sequences are recognized by ubiquitin ligases, which catalyze the attachment of a single ubiquitin molecule (31, 32). Ubiquitin molecules are subsequently added in a characteristic formation to create a polyubiquitin chain on the protein, and it is these ubiquitin chains that the protea-

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some uses to recognize proteins targeted for degradation (33). This mechanism, in part, allows tight regulation of protein degradation by the proteasome.

The 26S proteasome is a multiprotein complex composed of a 20S proteolytic core particle flanked at each end by the 19S regulatory complexes (34, 35). The 20S core is composed of four subunits that include two β and two α rings. Within each β ring, there are three active sites: a chymotrypsin-like site, a trypsin-like site, and a post-acidic or caspase-like site (also sometimes called peptidyl glutamyl peptide hydrolase-like site; Ref. 36). Of the three catalytic activities in the 20S core structure, the chymotrypsin-like site appears to have the most prevalent proteolytic activity (Fig. 1; Refs. 37, 38). The 19S complexes recognize the polyubiquitin chain on target proteins, recycle the polyubiquitin chain, and denature the target protein so that it can be moved into the catalytic chamber in the 20S core.

Bortezomib is a reversible but potent and selective inhibitor ($K_i = 0.6$ nM) of the chymotrypsin-like activity of the proteasome (39). Whereas the other proteolytic activities are not inhibited by bortezomib, inhibition of the chymotrypsin-like activity alone results in significant blockade of proteasomal protein degradation within the cell (40).

Androgen-Dependent and Androgen-Independent Prostate Cancer Growth. Androgens act through several pathways to support prostate cancer cell growth, and deprivation of androgens can induce cell death (1, 2). Androgens block Fas- or tumor necrosis factor α -induced apoptosis in androgen-dependent prostate cancer (LNCaP) cells by obstructing caspase activation in a manner that is both independent of the phosphatidylinositol 3'-kinase-PTEN-Akt pathway and without nuclear factor κ B (NF κ B) activation (41, 42). Androgen-independent growth can develop through several mechanisms.

AR mutations have been found in AIPC metastatic tumors and cell lines (43); these mutations (44, 45) can alter AR ligand specificity and lead to inappropriate AR activation (43). In the androgen-independent cell line MDAPa, mutations in the AR gene allowed binding to nonandrogen molecules such as cortisol, cortisone, and synthetic glucocorticoids (46).

Growth factor signaling pathways can activate AR independently of androgen via phosphorylation at specific AR sites (47). This point is particularly important, because the up-regulated paracrine and autocrine expression of growth factors and their cognate receptors coincides with tumor metastasis and androgen-independent growth. IL-6

serum levels are elevated in AIPC with advanced disease (48), and IL-6 transcription is inhibited by dihydrotestosterone through I κ B α in prostate cells (49), which suggests an inverse relationship between IL-6 and androgen in prostate cancer. The recent finding that IL-6 stimulation of androgen-responsive LNCaP cells results in phosphorylation of the AR and transcription of AR-driven promoters suggests a mechanism of cross-talk and redundancy between the IL-6 and AR growth-promoting pathways (50). Independent of their effects on the AR, other autocrine and paracrine factors are associated with progression of prostate cancer. IL-8 overexpression is correlated with angiogenesis and metastasis, as well as expression of matrix metalloproteinases and vascular endothelial growth factor, in an animal androgen-independent tumor model (51, 52). Vascular endothelial growth factor is also expressed in neuroendocrine-positive tumor cells of human prostate carcinoma specimens, and its expression is strongly associated with higher microvessel density, increasing tumor stage, and a less differentiated cellular morphology (53).

Constitutive activation of the transcription factor NF κ B may also be associated with more aggressive prostate cancers. Bortezomib-induced inhibition of I κ B α degradation by the proteasome would result in cytoplasmic NF κ B inhibition (through I κ B α binding) and inability of NF κ B to translocate to the nucleus and bind to the promoters of multiple genes. These NF κ B-targeted genes include proinflammatory cytokines (*i.e.*, IL-1, IL-6, and tumor necrosis factor- α), cell adhesion molecules (*i.e.*, vascular cell adhesion molecule, intercellular cell-adhesion molecule, and E-selectin), stress response enzymes (*i.e.*, cyclooxygenase 2, nitric-oxide synthase, and 5-lipoxygenase; Ref. 54), and antiapoptotic proteins (*i.e.*, inhibitor of apoptosis protein and the BCL-2 family; Refs. 55–58; Fig. 2). Many of these pathways are involved in tumor growth, angiogenesis, metastasis, and resistance to chemotherapy in both solid and hematological malignancies (59–63).

Blockade of NF κ B activity via the expression of a dominant-negative I κ B α construct resulted in the suppression of angiogenesis and prostate cancer cell invasion and metastasis (64). The *in vivo* expression of vascular endothelial growth factor, IL-8, and matrix metalloproteinase 9 was decreased in nude mice bearing xenografted tumors of human prostate cells transfected with the mutated I κ B α constructs (64). Additionally, NF κ B-dependent transcriptional activity was ~ 10 times higher in invasive PC-3 clones compared with less invasive lines, suggesting that NF κ B activity is associated with a highly metastatic phenotype (65). Constitutive NF κ B activity is also associated with resistance to the apoptosis-inducing effects of tumor necrosis factor α in AIPC cells (66). Muenchen *et al.* (67) found that both androgen-dependent (LNCaP) and androgen-independent (PC-3) prostate cancer cell lines could be made tumor necrosis factor α sensitive by transfecting them with a dominant-negative I κ B construct, resulting in reduction of IL-6 mRNA and protein expression. Although constitutive NF κ B activity alone is not sufficient for androgen independence and other cellular factors must be involved in mediating androgen independence in prostate cancer cells (68), it appears that NF κ B inhibition through proteasome inhibition or other methods remains a potential cellular target for cancer treatment (69, 70).

Mutations to tumor suppressor genes and oncogenes are also evident in established human androgen-independent and androgen-dependent prostate cancer cell lines, as well as primary prostate cancers and metastases (71–73). The human prostate cancer cell lines LNCaP, DU145, and PC-3 vary in their expression of important tumor suppressor genes such as *p53*, *retinoblastoma* (Rb), and phosphatase/tensin homologue deleted on chromosome 10 (*PTEN*), but no specific genotype has been assigned to cancer cells with androgen independence or dependence (Table 1). Inactivating mutations within

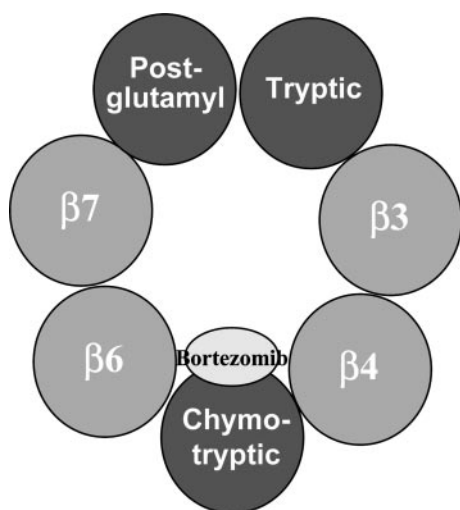


Fig. 1. Cross-sectional view of the 20S proteasome. Cross-section of the proteasome β ring, which contains the three active sites. Bortezomib binds and inhibits the chymotrypsin site. The function of the other four β subunits is unclear.

Fig. 2. Nuclear factor κ B ($NF-\kappa B$) activation pathway. Viruses, growth factors, antigens, radiation, or chemotherapeutic drugs activate signaling pathways that lead to the degradation of inhibitor of nuclear factor- κ B ($I\kappa B$) by the proteasome. Once released from $I\kappa B$ inhibition, $NF-\kappa B$ translocates to the nucleus to activate transcription of genes that protect the cell from apoptosis and promote cell growth and differentiation. The synthesis of growth factors, cell-adhesion molecules, angiogenesis factors, and antiapoptotic factors is increased.

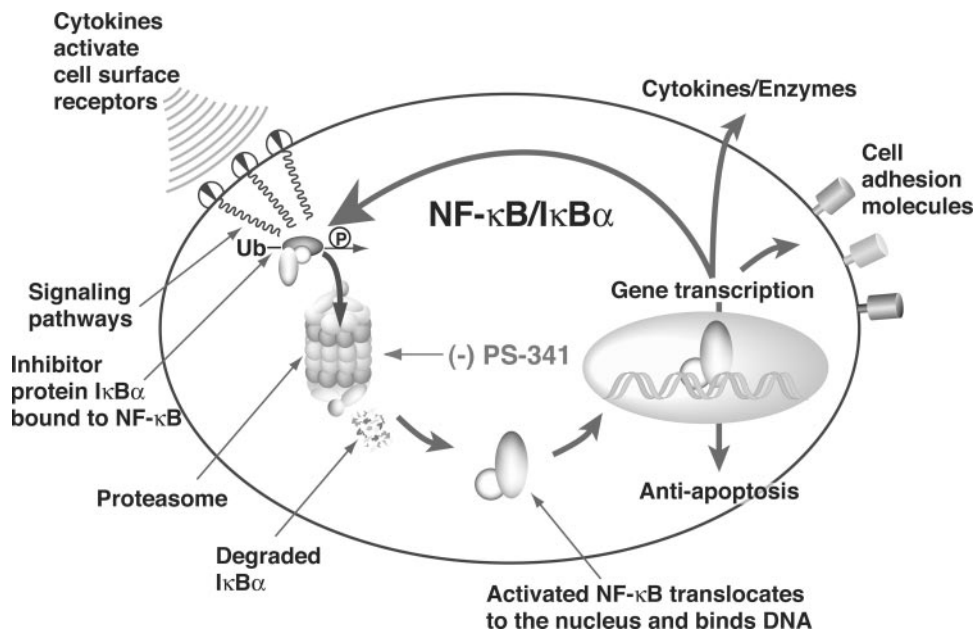


Table 1 Genotypes for common human prostate cancer cell lines

Cell line	Tissue of origin	Androgen-dependent	NF κ B ^a constitutive activation	Genotype		
				Rb	p53	PTEN
LNCaP	Lymph node	Yes	Yes	+/+	+/+	-/-
PC-3	Bone marrow	No	Yes	-/-	-/-	-/-
DU145	Brain tumor	No	Yes	+/+	-/-	+/+

^aNF κ B, nuclear factor κ B.

the *PTEN* tumor suppressor gene have been identified in primary prostate tumors, the prostate cell line DU145, and breast and brain tumors (71, 72). *PTEN* is a dual-specific phosphatidyl phosphatase that modulates cell-cycle progression and negatively regulates the phosphatidylinositol 3'-kinase/Akt (protein kinase B) pathway (74). *PTEN* loss is associated with up-regulation of Bcl-2 transcription and expression (64), loss of p27 expression, and tumor progression (75). The cyclin-dependent kinase inhibitor p27 has been identified as a prognostic factor in prostate cancer, and loss of p27 expression is correlated with advanced AIPC (76). *PTEN* inactivation also results in the constitutive activation of the phosphatidylinositol 3'-kinase/Akt pathway in both androgen-dependent and androgen-independent prostate tumor cells (75, 77–79). In turn, the activation of the Akt serine/threonine kinase modulates the expression of the AR in normal and prostate tumor cells (80).

Interestingly, the overexpression of the epidermal growth factor-like receptor tyrosine kinase Her-2/*neu* has been seen in prostate cancer (72) and in the primary tumors of patients who have undergone androgen ablation therapy (81). Her-2/*neu* overexpression was identified in primary tumors, and continued overexpression occurred in androgen-independent tumors that developed after androgen ablation therapy (82, 83). Like *PTEN* mutations, Her-2/*neu* overexpression activates Akt (84) and may trigger the AR transcriptional activity and promote growth in an androgen-independent manner (85). Her-2/*neu*-mediated Akt activation has also been associated with the adaptation to androgen independence by LNCaP cells (84), possibly through an NF κ B-mediated pathway (86). Akt phosphorylates $I\kappa B\alpha$, prompting degradation of $I\kappa B\alpha$ by the proteasome (28) and activating NF κ B (87), thereby protecting both androgen-dependent and androgen-independent cells from apoptosis. Thus, the transition to androgen-independent growth probably involves several different mechanisms

related to the perturbation of normal cell growth and tumor suppression in the cell, which may involve, in part, activation of AR, NF κ B, Akt, and other growth-signaling pathways (Fig. 3).

Promotion of Apoptosis by Bortezomib in Prostate Cancer Models. The proteasome is a hub for the regulation of many cellular signaling pathways, and therefore proteasome inhibition appears to induce apoptosis through a number of mechanisms (39). In prostate cancer, initial evidence indicates that sensitivity is independent of the requirements of the tumor for androgens. Experiments with bortezomib show that this agent induces apoptosis in androgen-dependent and androgen-independent prostate cancer cell lines (11–13). Deregulation of proteasomal function by inhibition of proteolysis induces apoptosis in tumor cells, including those without functional p53, PTEN, or p21 (13); cells overexpressing the antiapoptosis factor Bcl-2 (13, 88) or those that are resistant to conventional chemotherapies (89) are also sensitive. Additional studies examining the effects of proteasome inhibitors in animal tumor models support the proapoptotic activities demonstrated in cell culture systems (Table 2; Refs. 10, 11, 14, 15, 90–92). Frankel *et al.* (93) found that when androgen-inde-

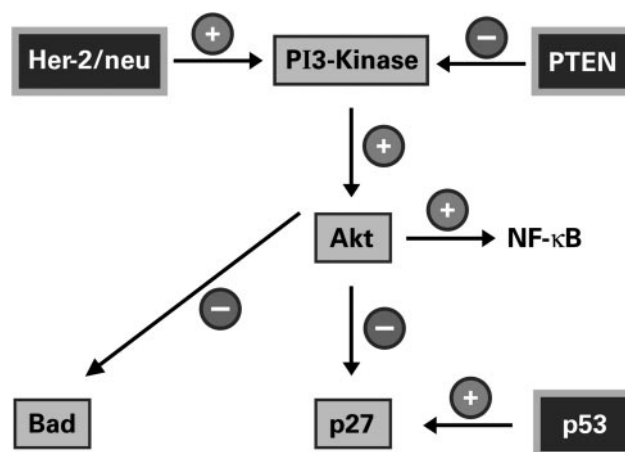


Fig. 3. Important cellular mediators of prostate cancer growth. PTEN and HER-2/*neu* regulate the phosphatidylinositol 3'-kinase (*PI3-Kinase*)/Akt signaling pathway, which, in turn, regulates the activity of important mediators of proliferation, survival, and apoptosis. *NF-κB*, nuclear factor κ B.

Table 2 Antitumor activities of bortezomib in solid tumor models

Cells	Animal model and dosing schedule	Antitumor activity
Human prostate cancer, PC-3 (11)	Athymic nude mice, weekly $\times 4$	60% decrease in tumor volume
Human MIA-PaCa-2 pancreatic cancer (10)	Athymic nude mice, twice weekly $\times 3.5$	75% growth inhibition with combination with bortezomib and gemcitabine versus 59% growth inhibition with gemcitabine alone
Human pancreatic cancer, BxPc-3 (15)	Athymic nude mice, weekly $\times 4$	89% growth inhibition with combination bortezomib and CPT-11 versus 43% with CPT-11 alone
Human LOVO colon cancer (14)	Athymic nude mice, twice weekly	94% decrease in tumor size with combination bortezomib and CPT-11 versus 48% with CPT-11 alone
Human LOVO colon cancer (90)	Athymic nude mice, 6 h before irradiation	7–41% increase in radiosensitivity with bortezomib leading to an 84% reduction in tumor volume
Squamous cell carcinoma: murine PAM-LY2 (91)	Balb/C SCID mice, three times weekly	50–80% dose-dependent decrease in tumor volume
Murine lung cancer and human breast cancer (92)	Lewis lung model, EMT-6 mouse model; daily treatment	Lewis lung model: growth delay and decreased number of lung metastases alone and in combination (5-fluorouracil, cisplatin, paclitaxel, or docetaxel); EMT-6: dose-dependent cytotoxicity alone and potentiated tumor cell killing with cyclophosphamide, allowing dose reduction

pendent DU-145 prostate cancer cells are grown in spheroid cultures, a system that mimics the low growth fractions of prostate cancer *in vivo* (94), bortezomib treatment caused cell death in a greater proportion than when the same prostate cancer cells were grown in monolayers. In mouse xenograft tumor models, athymic mice bearing PC-3 tumors were treated with four weekly *i.v.* doses of bortezomib, and dose-dependent tumor size reductions were observed. When the drug was injected into the tumor on 4 consecutive days, 70% of the animals had no detectable tumor at the end of the study (11).

Proteasome activity may also overcome resistance mechanisms in response to chemotherapy and radiation and may augment antitumor activity of these agents. Proteasome inhibition may increase the effectiveness of these therapeutic modalities by either restoring sensitivity to radiation or chemotherapy or by showing additive or synergistic effect, allowing for decreased dosing and potentially reduced toxicity. Cooperative antitumor activity has been seen with bortezomib and the topoisomerase I inhibitor CPT-11 (irinotecan; Refs. 14, 15). One known topoisomerase inhibitor resistance mechanism in cancer cells is the up-regulation of topoisomerase I/topoisomerase II proteasomal degradation (95). However, proteasome inhibition can block solid tumor resistance to topoisomerase II inhibitors (96). In addition, bortezomib pretreatment blocked CPT-11-mediated NF κ B DNA binding activity in colon cancer cells (14). This presumably occurs through the bortezomib-mediated stabilization of the proteasomal substrate I κ B α . These experiments suggest that bortezomib may contribute to increase chemosensitivity of tumors to topoisomerase I inhibitors and may be a promising second-line or adjuvant treatment for CPT-11, VP16, or other topoisomerase inhibitors.

Bortezomib also enhanced xenograft radiosensitivity in a mouse colon tumor model and blocked radiation-induced NF κ B DNA binding (90). Terminal transferase-mediated dUTP nick-end labeling analysis of the xenografts for apoptosis revealed that 4% of tumor cells were undergoing apoptosis in control mice, compared with 18% in mice treated with 6 Gy of radiation, 26% in mice treated with 1 mg/kg of bortezomib, and 72% in mice treated with both therapies (90). Pervan *et al.* (12) also showed enhanced radiosensitivity by proteasome inhibition in the TRAMP-C1 prostate cancer tumor model. Mice with TRAMP-C1 tumors received either bortezomib or placebo before radiation treatment. Animals receiving bortezomib treatment showed enhanced radiosensitivity and delayed tumor growth compared with controls. Considered together, these data suggest that bortezomib sensitizes cells to the effects of radiation and increases tumor cell apoptosis in mice treated with both therapies.

Other studies suggest that bortezomib may enhance the effectiveness of other classes of chemotherapy agents. Mice with Mia-PaCa-2 xenografts treated with gemcitabine had an average of 59% tumor size reduction, whereas mice receiving the combined bortezomib-

gemcitabine therapy saw an average tumor volume loss of 75%. Relatively high doses of bortezomib (1 μ M) induced apoptosis in the cells *in vitro* by reducing Bcl-2 transcription without an effect on Bax or Bak protein levels. However, low doses of the drug (10 nM) appeared to enhance gemcitabine-induced apoptosis. Schedule-dependent modulation has also been seen with bortezomib and taxanes (97–99). Cells treated with bortezomib before taxane administration had lower apoptotic indices (98), whereas taxane treatment preceding proteasome inhibition appeared to preserve the proapoptotic effects of taxanes in some cancer cells (97–99). Synergy between the two agents may not be present, because paclitaxel has been shown to induce phosphorylation and subsequent proteasomal degradation of Bcl-2 in NIH-OVCAR-3 cells (24). However, it is likely that this proposed abrogation of Bcl-2 phosphorylation by proteasome inhibitors is only a partial explanation for schedule-dependent synergy between bortezomib and taxanes, because Bcl-2 overexpression does not block bortezomib-induced apoptosis in prostate cells (13). We showed that treatment with either simultaneous bortezomib and paclitaxel or bortezomib followed by paclitaxel did not change the proapoptotic rate of PC-3 cells compared with bortezomib alone and was associated with moderate down-regulation of microtubule-associated protein 4 and marked down-regulation of β -tubulin with concomitant increase of unbound paclitaxel. By contrast, pretreatment of PC-3 cells with paclitaxel followed by bortezomib decreased their apoptotic rate by 53%, while preserving the up-regulated expression of both microtubule-associated protein 4 and β -tubulin, suggesting a cytoprotective effect of this treatment sequence (100).

Both prednisone (101) and dexamethasone (102, 103) have shown some antitumor activity in patients with AIPC, but the mechanisms responsible for their effects are not fully known. Dexamethasone treatment, however, is known to affect I κ B α and IL-6 regulation in multiple myeloma, a cancer that is highly sensitive to bortezomib (104), and both these mechanisms may be important in prostate cancer (48). In addition, bortezomib induced apoptosis in myeloma cells from patients with dexamethasone-resistant disease (89). In AIPC lines, dexamethasone inhibited proliferation, and this coincided with an increase of I κ B α protein, cytosolic accumulation of NF κ B, and decreased secretion of IL-6 (105).

Differential Sensitivity to Proteasome Inhibition. An important observation that appears to be consistent across many tumor types and proteasome inhibitors is that tumor cells seem to be more sensitive to proteasome inhibitors than normal cells (25). Myeloma cells isolated from patients are ~ 1000 times more sensitive to bortezomib-induced apoptosis than normal plasma cells of patients (89). Human leukemia cells that have been induced to differentiate are significantly less sensitive to the apoptotic effects of proteasome inhibitors than their rapidly proliferating precursors (106). Another line of evidence sug-

Table 3 Principal toxic effects during bortezomib treatment in androgen-independent prostate cancer (17)

Parameter	Any grade toxicity All cycles Patients (%)	Grade 3 toxicity ^a Cycle 1 Patients (%)
Parameter	n = 53	n = 53
Likely related to bortezomib		
Diarrhea	28 (53)	3 (6)
Fatigue	27 (51)	1 (2)
Constipation	25 (47)	1 (2)
Nausea	22 (42)	0
Vomiting	18 (34)	0
Weakness	14 (26)	2 (4)
Abdominal pain	13 (25)	0
Appetite decreased	12 (23)	0
Hypotension	10 (19)	3 (6)
Dizziness	8 (15)	0
Abdominal distension	7 (13)	0
Hypertension	6 (11)	1 (2)
Flatulence	6 (11)	0
Neuropathy	4 (8)	1 (2)
Likely unrelated to bortezomib		
Back pain	15 (28)	0
Catheter related complication	15 (28)	0
Bone pain	13 (25)	0
Pyrexia	13 (25)	0
Arthralgia	11 (21)	0
Headache	11 (21)	0
Urinary tract infection	11 (21)	0
Dyspnea	9 (17)	0
Anemia	8 (15)	0
Cardiac murmur	8 (15)	0
Hematuria	8 (15)	0
Hypomagnesemia	7 (13)	0
Edema lower limb	7 (13)	1 (2) (due to DVT)
Pain in limb	7 (13)	0
Nocturia	6 (11)	0
Rigors	6 (11)	0
Urinary frequency	6 (11)	0

^aNo grade 4 toxicity was observed during the study. DVT, Deep Vein Thrombosis.

gests that the induction of growth arrest of cancer cells in culture does not make cells less susceptible to bortezomib-induced apoptosis (107). Thus, it is unlikely that hypersensitivity of cancer cells to proteasome inhibition can be explained solely by their accelerated rate of division, because proteasome inhibition promotes apoptosis in slowly dividing prostate cancer cells grown in monolayer cultures and spheroids (93).

Early Clinical Experience with Bortezomib in AIPC. Seminal Phase I clinical trials have suggested that bortezomib has biological activity in advanced solid tumors and hematological malignancies with manageable toxicities (19). A Phase I study conducted at the M. D. Anderson Cancer Center at the University of Texas examined bortezomib in 53 patients with advanced solid tumors (17). Forty-eight of these patients were elderly men with AIPC. The median age of the patients was 66 years; 39 had received prior chemotherapy.

Patients were treated with i.v. bortezomib from 0.13 to 2.0 mg/m², once weekly for 4 weeks every 5 weeks. Thirty-one of 53 patients enrolled completed at least two cycles of bortezomib; 39 patients completed at least one cycle of treatment. In addition to monitoring toxicity, measurable disease response, serum IL-6, and prostate-specific antigen levels, 20S proteasome inhibition was measured in patients pre- and postdosing to examine possible correlations among bortezomib dose, proteasome inhibition, toxicity, and tumor response. The most frequent adverse events on this schedule included diarrhea, fatigue/weakness, constipation, nausea, vomiting, other gastrointestinal complaints, hypotension, dizziness, hypertension, and neuropathy. The dose-limiting toxicities included grade 3 diarrhea and grade 3 postural hypotension at the 2.0 mg/m² dose level (Table 3). In studies using a twice-weekly treatment regimen, peripheral sensory neuropathy was frequent; however, neuropathy was less common on the once-weekly schedule, occurring in 8% of patients (2% grade 3), although many patients had received potentially neurotoxic drugs previously.

Dose-related inhibition of whole-blood proteasome activity was observed, with most toxicity (diarrhea, hypotension, and fatigue) seen at doses (≥ 1.45 mg/m²) that yielded $\geq 70\%$ proteasome inhibition 1 h after treatment. The maximum tolerated dose in this schedule was 1.6 mg/m². Antitumor responses (measurable disease and prostate-specific antigen responses) were seen in 2 and 3 patients, respectively, among the 48 patients with AIPC. One of the 2 objective partial responses and all 3 of the prostate-specific antigen responses occurred at doses resulting in average 1-h postbortezomib whole-blood 20S proteasome inhibition of $\geq 70\%$, which also is associated with dose-limiting toxicity, suggesting a narrow therapeutic index with monotherapy in prostate carcinoma in this study. Proteasome inhibition was associated with decline in serum IL-6 levels, suggesting that bortezomib may be affecting NF κ B signaling and autocrine/paracrine mechanisms *in vivo*. Additional studies of bortezomib in patients with prostate cancer are warranted, and we are focusing on combination treatments with cytotoxic agents that may reverse resistance to chemotherapy.

Two additional single-agent Phase I trials of bortezomib, one in solid tumors (19) and another in hematological malignancies (16), have been published that support additional studies of this agent for the treatment of cancer. Whereas these studies were not designed to assess efficacy, clinically significant responses were observed. Bortezomib has demonstrated significant biological activity in a Phase II study of relapsed and refractory myeloma patients (108) and has received United States Food and Drug Administration approval for patients with relapsed or refractory multiple myeloma who have received at least two prior therapies and have progressed on their last

Table 4 Clinical activity of single-agent bortezomib

Tumor type	Dose schedule (intravenous bolus)	Activity
Relapsed, refractory multiple myeloma (108)	1.3 mg/m ² twice weekly every 3 weeks for up to 8 cycles	35% overall response rate (complete response + partial response + minimal response) by European Blood and Marrow Transplantation criteria (110); 18% clinical remission rate by Southwest Oncology Group criteria
Malignant lymphoma (111, 112)	1.5 mg/m ² twice weekly every 3 weeks	Promising activity, particularly in mantle cell lymphoma, in patients with relapsed disease (studies are ongoing)
Metastatic renal cell carcinoma	1.3–1.5 mg/m ² twice weekly every 3 weeks	3 durable partial responses among 32 treated patients in an ongoing trial (113); 1 partial response in 21 evaluable patients (114)
Non-small cell lung carcinoma	1.5 mg/m ² twice weekly every 3 weeks (115)	1 partial response and 2 patients with stable disease among 8 evaluable patients (115); 1 partial response in 8 patients enrolled in a dose-escalation study (19)
Metastatic androgen-independent prostate cancer	0.13–2.0 mg/m ² weekly \times 4 wks q 5 wks	2 radiographic partial responses in lymph nodes and 3 PSA partial responses among 48 treated patients (17). All responses occurred at doses resulting in average 1-h post-bortezomib whole-blood 20S proteasome inhibition $\geq 70\%$, which was also associated with a decline in serum interleukin 6 levels

therapy (109). Additional trials studying bortezomib as monotherapy and in combination with other antineoplastic agents are ongoing in multiple myeloma and in other types of malignancy. Most are Phase I exploratory studies, and promising results are beginning to emerge (Table 4; Refs. 108, 110–115). A Phase III randomized trial of the treatment of multiple myeloma with bortezomib monotherapy *versus* dexamethasone was halted recently at the interim analysis because of significantly greater efficacy in the bortezomib arm. Study analyses are ongoing.

Conclusion

The proteasome plays a central role in regulation of the cell cycle, proliferation, cell death, angiogenesis, metastasis, and resistance to chemotherapy and radiation therapy. Results from cell culture systems, animal tumor models, and early clinical studies suggest that proteasome inhibition represents a new therapy target for human cancers, including AIPC. Bortezomib is the first agent of this novel class to enter clinical trials, and antitumor activity has been reported from preclinical investigations in a variety of tumor models. In some diseases, like multiple myeloma, antitumor activity is significant even as a single agent, and bortezomib has been approved for the treatment of patients with multiple myeloma who have received at least two prior therapies and have demonstrated disease progression on their last therapy. In AIPC, bortezomib seems to have weak single agent antitumor activity. Preclinical models suggest synergy with some conventional chemotherapeutic agents with activity against AIPC. Additional studies are under way to additionally examine the role of bortezomib in combination with chemotherapy in AIPC as well as other cancers in which the proteasome has an active role in transformation, chemoresistance, and disease progression.

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