Review

The Proteasome as a Target for Cancer Therapy

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Abstract

The proteasome is a multicatalytic proteinase complex responsible for the degradation of most intracellular proteins, including proteins crucial to cell cycle regulation and programmed cell death, or apoptosis. In preclinical cancer models, proteasome inhibitors induce apoptosis, have in vivo antitumor efficacy, and sensitize malignant cells and tumors to the proapoptotic effects of conventional chemotherapeutics and radiation therapy. Interestingly, transformed cells display greater susceptibility to proteasome inhibition than nonmalignant cells. Therefore, proteasome inhibition holds promise as a novel approach to the treatment of cancer. Inhibitors of the proteasome impact on cells in part through down-regulation of nuclear factor κB, but also through modulation of cell cycle proteins and other pro- and anti-apoptotic pathways. Bortezomib (VELCADE; formerly PS-341), the first such inhibitor to undergo clinical testing, has demonstrated impressive antitumor activity and manageability in Phase I and II trials both as a single agent, and in combination with other drugs. It has been approved recently by the Food and Drug Administration for therapy of patients with multiple myeloma who have received at least two prior regimens and progressed on the last of these. Ongoing preclinical evaluations of the mechanisms that underlie the antitumor effects of proteasome inhibitors, and clinical trials in a variety of tumor types, will allow additional refinement of the role these agents will play in cancer therapy. Below we discuss the rationale behind targeting the proteasome for cancer therapy, and review the preclinical and clinical data on proteasome inhibitors alone, and in combination with conventional chemotherapeutics.

Introduction

The Ubiquitin-Proteasome Pathway (UPP). Precise control of protein turnover is essential to cellular survival. In eukaryotes, the majority of protein degradation occurs through the UPP, which consists of the ubiquitin-conjugating system and the proteasome. The proteolytic activities of the proteasome are located within a 20S multisubunit structure consisting of four stacked rings arranged around an inner channel. This macromolecule by itself is involved in turnover of some proteins, and also forms the active proteolytic core of up to three other complexes (Fig. 1). When capped by the 19S regulatory complex at each end, the 20S complex forms the core of the 26S proteasome, the major extralysosomal mediator of protein degradation. The ubiquitin-conjugating system targets proteins for degradation by the covalent attachment of multiple ubiquitin (Ub) chains (Fig. 2). Ubiquitination is mediated by the sequential action of an E1 Ub-activating enzyme, an E2 Ub-conjugating enzyme, and an E3 Ub-ligase. Once Ub-tagged, proteins bind to subunits in the 19S regulatory cap of the proteasome, where they are deubiquitinated and unfolded in an energy-dependent manner. These are then fed into the catalytic inner chamber of the 20S complex, which generates peptides of 3–22 amino acids in size (1–3).

The Proteasome and Apoptosis. Programmed cell death, or apoptosis, is necessary to the survival of all multicellular organisms through roles in normal growth and development. The discovery that a family of proteases, the caspases, mediated the execution of apoptosis generated interest in a possible role of the proteasome in this process. Studies were facilitated by the advent of pharmacological inhibitors of the proteasome, including synthetic peptidyl aldehydes and the bacterial compound lactacystin. Perhaps surprisingly, incubation of a human monoblast cell line with lactacystin led to apoptosis, demonstrating that proteasome inhibitors possess antitumor activity (4). Fujita et al. (5) provided the first evidence that proteasome inhibitors sensitize tumors to conventional therapies by showing that treatment of U937 cells with lactacystin alone did not induce apoptosis, but potentiated tumor necrosis factor-mediated cell death. Shortly thereafter, the first in vivo demonstration of the antitumor activity of proteasome inhibitors was reported, in which a peptidyl aldehyde induced tumor growth delay in a murine xenograft model of Burkitt’s lymphoma (6). An additional advance was made in the synthesis of the proteasome inhibitor PS-341, since renamed bortezomib, a dipetide boronic acid analogue that possesses pharmacological characteristics better suited for clinical testing in patients (7). Bortezomib demonstrated broad and unique antitumor activity in a National Cancer Institute tumor cell line screen, as well as in several murine xenograft models. These findings have paved the way for many exciting preclinical and clinical studies of the proteasome as a target for cancer therapy.
Chronic lymphocytic leukemia (CLL) cells were nontransformed human lymphoblasts (6). Patient-derived induced apoptosis than primary fibroblasts or immortalized, were up to 40-fold more susceptible to proteasome inhibition. For example, fibroblasts demonstrated a selective susceptibility of transformed cells to seem incompatible with life. Remarkably, several studies have – protein degradation (1–3), and its inhibition would therefore suggest that deregulated UPP function may play a direct role in the ubiquitin-proteasome pathway, as well as in ubiquitin-independent proteolysis.

**Differential Susceptibility of Transformed Cells**

The proteasome is responsible for >80% of intracellular protein degradation (1–3), and its inhibition would therefore seem incompatible with life. Remarkably, several studies have demonstrated a selective susceptibility of transformed cells to proteasome inhibition. For example, fibroblasts transformed with ras and c-myc, and lymphoblasts transformed with c-myc, were up to 40-fold more susceptible to proteasome inhibitor-induced apoptosis than primary fibroblasts or immortalized, nontransformed human lymphoblasts (6). Patient-derived chronic lymphocytic leukemia (CLL) cells were ~10-times more sensitive to lactacystin than normal lymphocytes (8). CD34+ hematopoietic progenitor cells from chronic myeloid leukemia patients were roughly three times more susceptible to proteasome inhibitor-mediated apoptosis than cells from normal volunteers (9). Malignant plasma cells from multiple myeloma patients were 20–40-times more sensitive to bortezomib-mediated apoptosis than blood mononuclear cells (10). Finally, leukemic stem cells from acute myeloid leukemia patients were more susceptible to the peptidyl aldehyde MG132 than normal hematopoietic stem cells (11).

Whereas the molecular basis of this differential susceptibility remains elusive, and may differ from one model system to another, several interesting possibilities exist. Drexler (12) showed that HL60 leukemia cells underwent proteasome inhibitor-mediated apoptosis when they were proliferating, but not when they were induced to differentiate into macrophages. Similarly, Lopes et al. (13) found that actively proliferating Rat-1 fibroblasts underwent apoptosis when exposed to proteasome inhibitors, but not when they were quiescent. However, the same investigators noted that pheochromocytoma cells underwent apoptosis regardless of whether they were proliferating or had undergone differentiation. Similarly, patient-derived CLL cells, which are predominantly in the G0 phase of the cell cycle, can undergo apoptosis (14). Thus, whereas actively dividing cells appear to be more susceptible to proteasome inhibition than quiescent cells, other elements must be at play.

One factor that may contribute is the cyclin-dependent kinase inhibitor p27Kip1. An et al. (15) found that the induction of apoptosis in SV40-transformed fibroblasts correlated with the accumulation of p27. In contrast, the parental normal fibroblast cell line was resistant to proteasome inhibition and did not accumulate significant levels of p27.

Strong evidence exists supporting a role for the deregulation of various UPP functions in malignant transformation. For example, CLL cells have 3-fold higher levels of chymotrypsin-like proteasome activity than normal lymphocytes (16). Increased proteasomal degradation of p27 leading to low levels of this cyclin-dependent kinase inhibitor confers a worse prognosis on patients with colon cancer and mantle cell lymphoma (17, 18). Similarly, decreased levels of the proapoptotic protein Bax are associated with increased proteasome activity and higher Gleason scores in prostate cancer (19). Recent global gene expression profiling of patient-derived myeloma cells has provided some additional interesting insights (20). Hierarchical clustering identified four distinct patient subgroups, and one of several genes differentially expressed in the poor prognostic subgroup was POH1, a 26S proteasome-associated protein. POH1 overexpression confers resistance to several chemotherapeutics, including doxorubicin, and to UV radiation (21). Another interesting finding was that, compared with normal plasma cells, the CDC34 gene transcript was strongly up-regulated in myeloma samples. CDC34 is part of an E2/E3 complex responsible for ubiquitination of a number of important substrates, including p27. More recently, pharmacogenomic analysis of patient samples from a Phase II trial of bortezomib in multiple myeloma suggested that transcription of culin4A, which functions as part of an E3 Ub ligase complex, may be a predictor of response to therapy (22). While speculative, the above evidence suggests that deregulated UPP function may play a direct role in
The ubiquitin-proteasome protein degradatory pathway. Proteins destined for degradation through the ubiquitin-proteasome pathway are first labeled by the ubiquitin conjugation pathway. An E1 ubiquitin-activating enzyme activates ubiquitin via the generation of a thiol ester bond between the COOH-terminal glycine on ubiquitin and an internal E1 cysteine residue. One of several known E2 ubiquitin-conjugating enzymes transfers the activated ubiquitin moiety to an E3 family member, of which there are many. The E3 ubiquitin ligase subsequently mediates the covalent attachment of multiple ubiquitin moieties to the protein destined for degradation by the 26S proteasome. This target protein is recognized through its ubiquitin tag by the 19S cap structure of the 26S proteasome, isopeptidases cleave off the ubiquitin moieties, which are recycled, and the target protein enters the proteasome. Inside the proteasome the target is cleaved into oligopeptides, which exit the proteasome, and may be subject to additional degradation into amino acids. Both the 26S and 20S proteasomes can also function in ubiquitin-independent proteolysis (data not shown).

Molecular Targets of Proteasome Inhibitors

Given the myriad of proteasome substrates, it is not surprising that inhibition of its function affects many pathways. Several important proteins that are regulated by the proteasome include the inhibitor of nuclear factor κB (NFκB; IκB), the tumor suppressor p53, the cyclin-dependent kinase inhibitors p21 and p27, and the proapoptotic protein Bax (Table 1). Accumulation of these substrates on proteasome inhibition leads to decreased NFκB-dependent transcription of genes crucial to the promotion of tumorigenesis, increased p53-mediated transcription of genes important to apoptosis and negative regulation of the cell cycle, p21- and p27-mediated induction of cell cycle arrest, and promotion of apoptosis via the inhibition of Bcl-2 by Bax. Proteasome inhibitors also down-regulate signaling through the p44/42 mitogen-activated protein kinase (MAPK), a pathway crucial to the promotion of tumorigenesis in a number of model systems. A detailed description of all of the potential targets of proteasome inhibitors is beyond the scope of this review; however, below we concentrate on some of the better-studied mechanisms through which proteasome inhibitors achieve their antitumor activity. In addition to these, other pathways that may also be of importance, but for which data are still somewhat preliminary, are included in Table 1.

NFκB. The NFκB transcription factor family is an important modulator of immune and inflammatory responses. More recent evidence has accumulated supporting their role in tumorigenesis through induction of cell proliferation, suppression of apoptosis, enhancement of tumor cell invasiveness and metastasis, and induction of angiogenesis (reviewed in Ref. 23). The UPP plays a critical role in regulation of the NFκB family of transcription factors in that NFκB1 and -κB2 are processed into their mature forms through a UPP-dependent pathway. In addition, IκB binds NFκB in the cytoplasm, thereby blocking its nuclear translocation. A variety of stimuli lead to the phosphorylation of critical serine residues on IκB, targeting it for ubiquitination and degradation by the proteasome, which then allows NFκB to enter the nucleus and mediate transcription (23). Expression of a “super-repressor” IκB in which these serines are mutated to alanines, and cannot therefore be phosphorylated, prevents proteasome-mediated degradation of IκB and sequesters NFκB in the cytoplasm, thereby mimicking the impact of proteasome inhibitors on this pathway.

Given the role of the proteasome in IκB degradation, proteasome inhibitors might induce apoptosis by stabilizing cytoplasmic IκB and blocking NFκB nuclear translocation. In support of this, infection of a myeloma cell line with the super-repressor IκB led to increased apoptosis (24). Similarly, treatment of myeloma cell lines and patient samples with bortezomib resulted in growth inhibition, even in drug-resistant cell lines. Growth inhibition and apoptosis correlated with IκB stabilization and decreased NFκB activity. However, other potential targets of proteasome inhibition were impacted upon as well, including p21Cip1 and p27Kip1 (10). In a murine xenograft model of head and neck squamous cell carcinoma, expression of an IκB super-repressor, or treatment with bortezomib, led to inhibition of tumor growth and angiogenesis, correlated with inhibition of NFκB activity, and down-regulation of growth- regulated oncogene α and vascular endothelial growth factor, NFκB-dependent proangiogenic cytokines (25). It is also interesting to note that, given the role of NFκB in inflammatory responses, proteasome inhibitors hold promise as anti-inflammatory agents (reviewed in Ref. 26).

Not all studies, however, have confirmed a role for NFκB attenuation as a mechanism of proteasome inhibitor-induced cell death. Treatment with lactacystin sensitized B-cell lymphoma cells to radiation-mediated apoptosis, but inhibition of NFκB activity was not demonstrated (27). In another study, treatment of CLL cells with lactacystin did not lead to cytoplasmic accumulation of IκB or nuclear loss of NFκB (16). Given the broad number of substrates of the proteasome, it seems unlikely that inhibition of NFκB is the sole mechanism of antitumor activity.
p44/42 MAPK. Like NFκB, the p44/42 MAPK pathway plays important roles in cell proliferation, suppression of apoptosis, and angiogenesis through activation of a signaling cascade that ultimately phosphorylates p44 and p42 (reviewed in Refs. 28–30). Through additional phosphorylation events, these kinases modulate the activity of several important signaling molecules and transcription factors, including c-Myc and NFκB itself. The p44/42 pathway is important in a variety of tumors, including both hematological malignancies such as leukemias and solid tumors such as breast cancer (reviewed in Refs. 31, 32). In the latter, where p44/42 has been particularly well studied, signaling through the receptor tyrosine kinase, Her2, is mediated in part through p44/42 MAPK. Overexpression of Her2 portends a worse prognosis in breast cancer patients, and increased p44/42 activity may be associated with poorer patient outcomes independent of Her2 expression. Proteasome inhibitors act in part through down-regulation of the p44/42 MAPK signaling cascade. Treatment of myeloma cells with bortezomib decreased activation of p44/42, which correlated with inhibition of myeloma growth (10). More extensive studies of the impact of proteasome inhibitors on p44/42 have been performed in breast cancer model systems. Treatment of breast cancer cell lines with proteasome inhibitors led to a loss of dually phosphorylated, active p44/42 MAPK (33). Diphosphorylation of p44/42 correlated with transcriptional induction of the MAPK phosphatases 1 and 2. Studies with pharmacological agents and dominant-negative mutant constructs inhibiting p44/42 signaling enhanced apoptosis, whereas activation of p44/42 significantly opposed apoptosis.

The p53 Tumor Suppressor. Degradation of the p53 protein occurs predominantly through the UPP (reviewed in Ref. 34), and proteasome inhibitors may exert some of their effects through p53 accumulation. Increased p53 levels would increase transcription of cell cycle targets such as p21 and the proapoptotic protein Bax. Many studies have demonstrated increased levels of p53 or its targets upon proteasome inhibition. Direct evidence that p53 plays a role came from an early study on Rat-1 fibroblasts (13), in which expression of a dominant-negative p53 attenuated proteasome inhibitor-induced apoptosis. Comparable studies in other model systems have been confirmatory of a role for p53 accumulation. It is important to note, however, that whereas p53 is an attractive and logical candidate contributing to proteasome inhibitor-mediated apoptosis, many studies have shown that proteasome inhibition activates apoptosis regardless of the p53 status of a cell (e.g., Refs. 12, 35). This is likely a testament to the multiple molecular targets of these agents. In addition, because many p53 targets such as p21 and Bax are themselves degraded through the UPP, they would accumulate in response to proteasome inhibition even in the absence of p53.

Cyclin-Dependent Kinase Inhibitors p21 and p27. p21 and p27, members of the Cip/Kip family of cyclin-dependent kinase inhibitors, stop cell cycle progression at the G1–S phase by binding to, and inactivating, a number of cyclin/cyclin-dependent kinase complexes. The UPP plays a crucial role in the turnover of both proteins. Therefore, proteasome inhibitors increase levels of p21 and p27 by decreasing proteasomal degradation of both proteins, and by increasing p53-mediated transcription of p21. Evidence that p21 plays an important role in the antitumor effect of proteasome inhibitors is largely circumstantial. Many investigators have shown that p21 accumulates on proteasome inhibition, but others have found that p21 is dispensable to inhibitor-induced cell death (36). Perhaps more interesting is the role that p27 may play. As described above, down-regulation of p27 activity plays an important role in the oncogenesis of a number of tumor types, and low levels of p27 in some tumors are the result of increased proteasome-mediated degradation. The fact that p27 up-regulation is directly involved is supported by the finding that introduction of antisense p27 oligonucleotides into an oral squamous cancer cell line partially blocked proteasome inhibitor-mediated apoptosis (37). Thus, at least in some systems, accumulation of p21, p27, or both may contribute to cell-cycle arrest of tumor cells and eventual apoptosis.

Bax. Whereas the proteasome modulates apoptosis indirectly through NFκB-dependent transcription of a number of anti-

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**Table 1 Molecular targets of proteasome inhibitors**

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequelae of proteasome inhibition</th>
<th>Contribution to antitumor effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFκB&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stabilization of IkB, which inhibits nuclear translocation of NFκB</td>
<td>Decreases NFκB-dependent transcription of genes important in tumor cell survival, proliferation, invasion and metastasis, and angiogenesis</td>
</tr>
<tr>
<td>p53</td>
<td>Accumulation of p53 protein by inhibition of proteasome-mediated p53 degradation</td>
<td>Increases p53-dependent transcription of cell cycle inhibitors (p21) and proapoptotic factors (Bax)</td>
</tr>
<tr>
<td>p21 and p27</td>
<td>Accumulation of both p21 and p27, and increased transcription of p21 through accumulation of p53</td>
<td>Induces G1/S cell cycle arrest and apoptosis</td>
</tr>
<tr>
<td>Bax</td>
<td>Accumulation of Bax by inhibition of proteasome-mediated Bax degradation and through increased p53-mediated transcription</td>
<td>Increases Bax interaction with Bcl-2 and Bcl-xL, promoting release of mitochondrial cytochrome c and apoptosis</td>
</tr>
<tr>
<td>p44/42 MAPK</td>
<td>Transcriptional activation of the MKP-1 phosphatase, leading to p44/42 dephosphorylation and inactivation</td>
<td>Down regulates p44/42-dependent cell proliferation and survival signals, and possibly angiogenesis</td>
</tr>
<tr>
<td>tBid (62)</td>
<td>Accumulation through decreased proteasomal degradation</td>
<td>tBid induces conformational changes in Bax, promoting mitochondrial release of cytochrome c</td>
</tr>
<tr>
<td>Smac/Diablo (63)</td>
<td>Accumulation through decreased proteasomal degradation</td>
<td>Smac and Diablo bind to and inhibit members of the XIAP protein family</td>
</tr>
</tbody>
</table>

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<sup>a</sup> NFκB, nuclear factor κB; IkB, inhibitor of nuclear factor κB; MAPK, mitogen-activated protein kinase; Diablo, direct IAP binding protein with low pI; Smac, Second mitochondria-derived activator of caspase; XIAP, X-chromosome-linked inhibitor of apoptosis.
apoptotic genes, the UPP likely plays a direct role as well by degrading important proapoptotic proteins. Upon dephosphorylation, Bax interacts with and inhibits the antiapoptotic proteins Bcl-2 and Bcl-xL in mitochondria, leading to cytochrome c release and activation of the caspase cascade. Li and Dou (19) demonstrated recently that Bax levels are regulated by proteasome-mediated degradation. Apoptosis in Bcl-2 overexpressing Jurkat cells treated with lactacystin correlated with the accumulation of mitochondrial-associated Bax. Other studies have shown that bortezomib can induce Bcl-2 phosphorylation and cleavage in association with G2-M phase arrest (38), supporting the possibility that several mechanisms may be responsible for the ability of these agents to abrogate the antiapoptotic effects of Bcl-2.

Proteasome Inhibitors Overcome Resistance to Standard Therapies

Both de novo and acquired resistance to chemotherapy and radiation therapy limit the effectiveness of many treatments currently available to patients, and proteasome inhibitors may impact on both mechanisms (Table 2). Strong evidence exists supporting a role for NFκB as part of inducible chemoresistance (23), whereby proapoptotic stimuli also activate antiapoptotic survival pathways. For example, treatment of fibrosarcoma cells with tumor necrosis factor α, ionizing radiation, or the anthracycline daunorubicin led to nuclear NFκB accumulation. Inhibition of NFκB nuclear translocation with an IkB super-repressor dramatically potentiated apoptosis to these agents. The camptothecin CPT-11 also activated NFκB in fibrosarcoma cells, and whereas xenografts were unresponsive to CPT-11 or the super-repressor IkB alone, the combination of the two led to significant tumor growth inhibition (39).

Down-regulation of NFκB likely plays an important role in the ability of proteasome inhibitors to abrogate drug resistance. Using a colon cancer xenograft model, Cusack et al. (40) demonstrated that the combination of bortezomib and CPT-11 was more effective than either agent alone. Tumor regression correlated with synergistic induction of apoptosis. Treatment with CPT-11 alone activated NFκB, whereas the combination therapy led to complete NFκB inhibition. Proteasome inhibitors may also play a role as radiation sensitzers. In a mouse colon cancer xenograft model, tumors were treated with a single dose of radiation or bortezomib alone or in combination. The post-treatment to pretreatment tumor volume ratios, and the levels of apoptosis, suggested a synergistic interaction between the therapies, and treatment with the IkB super-repressor led to similar results (41). Ma et al. (42) showed recently that treatment of myeloma cell lines resistant to melphan, mitoxantrone, and doxorubicin with a subtoxic dose of bortezomib sensitized them to treatment with these agents, which became cytotoxic at 10,000–100,000-fold lower concentrations in the presence of bortezomib. NFκB activity in these resistant cell lines was higher than that of nonresistant myeloma cell lines. β1 integrin-mediated adhesion of myeloma cells to fibronectin also plays an important role in de novo drug resistance. Landowski et al. (43) showed that treatment with proteasome inhibitors attenuated adhesion-mediated drug resistance and nuclear NFκB activity.

Proteasome inhibitors may down-regulate other resistance pathways as well. Several groups of chemotherapeutics, most notably the taxanes and anthracyclines, activate signaling through the p44/42 MAPK pathway, and in some model systems this has been shown to be antiapoptotic. The ability of proteasome inhibitors to induce MKPs might indicate that these agents would be useful in enhancing taxane- or anthracycline-mediated apoptosis by down-regulating p44/42 MAPK activation. In support of this possibility, early evidence from our laboratory shows that the proteasome inhibitor bortezomib blocks doxorubicin-mediated activation of p44/42 MAPK in a murine xenograft model of breast cancer. Furthermore, this correlates with the ability of the combination therapy to enhance apoptosis and antitumor efficacy.3

Anthracyclines and the epipodophyllotoxins work in part by inhibiting topoisomerase II (TopII), stabilizing the TopII-

Table 2  Chemo/radiosensitization by proteasome inhibitors

<table>
<thead>
<tr>
<th>Target</th>
<th>Sequelae of proteasome inhibition</th>
<th>Contribution to antitumor effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFκBa</td>
<td>Inhibits nuclear translocation of NFκB that would ordinarily be stimulated by many chemotherapeutics and radiation</td>
<td>Blocks NFκB-mediated transcription of antiapoptotic genes such as Bcl-1-2, Bcl-xL, XIAPs</td>
</tr>
<tr>
<td>p44/42 MAPK</td>
<td>Inhibits activation of p44/42 that is mediated by several chemotherapeutics, including taxanes</td>
<td>Down regulates p44/42-dependent survival signals</td>
</tr>
<tr>
<td>P-gp</td>
<td>Inhibits processing of P-glycoprotein precursors, resulting in accumulation of immature forms</td>
<td>May prevent efflux of cytotoxic agents through the P-gp pump, increasing their intracellular concentrations</td>
</tr>
<tr>
<td>TopI</td>
<td>Stabilizes TopI, of which the degradation is stimulated by agents derived from camptothecins, such as irinotecan</td>
<td>May stabilize the TopI-DNA cleavable complex, prevent its repair and DNA religation, promoting apoptosis</td>
</tr>
<tr>
<td>TopIIα</td>
<td>Accumulation of TopII, of which the degradation in some tumors is increased as a mechanism of resistance</td>
<td>Increased abundance of the target for TopII inhibitors, such as anthracyclines and epipodophyllotoxins</td>
</tr>
<tr>
<td>Genotoxic stress response</td>
<td>Decreased transcription of the DNA repair machinery</td>
<td>Sensitization of tumors to DNA-damaging agents (alkylating agents, anthracyclines, epipodophyllotoxins, platinum-based compounds)</td>
</tr>
</tbody>
</table>

a NFκB, nuclear factor κB; MAPK, mitogen-activated protein kinase; P-gp, P-glycoprotein; TopI, topoisomerase I; TopIIα, topoisomerase IIα.

3 R. Orlowski, unpublished observations.
DNA cleavable complex and inhibiting religation of single- and double-stranded DNA breaks. Evidence suggests that tumors may develop resistance to these agents through the down-regulation of their preferred target, TopIIα, a proteasome substrate. Therefore, proteasome inhibitors might overcome resistance to TopII inhibitors by up-regulating levels of TopIIα. Consistent with this possibility, Ogiso et al. (44) demonstrated that TopII depletion in colon and ovarian cancer cells occurred in response to glucose deprivation and hypoxia, two conditions encountered frequently in tumors. Lactacystin prevented down-regulation of TopIIα in colon cancer cells deprived of glucose and oxygen, potentiating the effects of etoposide and doxorubicin, but not of other agents. Using a colon cancer xenograft model, these investigators demonstrated increased tumor inhibition with the combination treatment of etoposide and lactacystin. Interestingly, the camptothecins, which target topoisomerase I, induce UPP-dependent topoisomerase I degradation, suggesting that proteasome inhibitors may potentiate the activity of these agents not only by down-regulating NFκB activation, but also by stabilizing topoisomerase I (45).

Perhaps the best-known mechanism of chemotherapy resistance is P-glycoprotein (P-gp), a cell surface transporter that functions as an efflux pump for intracellular toxins. Several classes of drugs, including the anthracyclines, Vinca alkaloids, and epipodophyllotoxins, are P-gp substrates. Interestingly, proteasome function is required for the normal maturation of P-gp. Proteasome inhibition leads to accumulation of immature P-gp that is incapable of transporting drugs out of cells efficiently. Thus, whereas it has not been tested directly, proteasome inhibitor-mediated blockade of P-gp maturation may function to abrogate resistance to P-gp substrates (46).

Recent studies have suggested that proteasome inhibitors synergize with DNA damaging agents by inhibiting the transcription of genes involved in DNA damage repair. Mitsiades et al. (47) showed that subtoxic concentrations of bortezomib sensitized chemotherapy-resistant myeloma cell lines and patient samples to doxorubicin and melphalan. Gene expression profiling demonstrated down-regulation of a number of transcripts involved in DNA repair. The authors proposed that the abrogation of resistance to melphalan and doxorubicin was mediated in part by transcriptional down-regulation of the protective response to genotoxic stress.

It should be noted, however, that proteasome inhibition has not overcome chemoresistance in all cases. For example, bortezomib was unable to reverse breast cancer cell resistance to cyclophosphamide or cisplatin in vitro (48). These findings point to the need for additional elucidation of the mechanisms involved in the action of these agents, so that their optimal use can be identified.

Clinical Data
Given the broad spectrum of activity of proteasome inhibitors in in vitro and in vivo models, interest in the proteasome as a target for cancer therapy has increased dramatically. To date, the first and only such inhibitor to have entered clinical trials is bortezomib, a peptide boronic acid derivative, which offers several advantages over some of the other available proteasome inhibitors. Peptidyl aldehydes such as MG132 are less specific than bortezomib in that they inhibit serine and cysteine proteases in addition to the proteasome, and also have poor stability and bioavailability. The bacterial product lactacystin, whereas more proteasome specific, irreversibly blocks several of the proteolytic activities of the proteasome, whereas bortezomib reversibly inhibits the chymotrypsin-like activity. These unique features of bortezomib may make it easier to administer in the in vivo setting and also may decrease its toxicity. Over 90% of bortezomib is cleared from the plasma compartment within 15 min, and therefore to better guide dosing in Phase I clinical trials a bioassay was developed that allows measurement of proteasome function in whole blood or tumor samples. Using this assay, investigators showed that normal proteasome activity is restored 2–3 days after the administration of bortezomib i.v. They were also able to demonstrate drug delivery to all of the tissues except the central nervous system, eyes, and testes, suggesting that therapeutic benefits from treatment of tumors in these locations may be limited in the setting of, for example, an intact blood-brain barrier.

Single Agent Phase I Studies with Bortezomib. Several Phase I clinical trials have evaluated bortezomib for the treatment of refractory and relapsed solid tumors and hematological malignancies. In the first published data, 43 patients with solid tumors were treated with bortezomib given as an i.v. bolus injection on days 1, 4, 8, and 11 of a 3-week cycle (49). In preclinical studies it was found that more frequent therapy increased toxicity, indicating the need for some recovery of proteasome function between dosing for normal cellular homeostasis. Dose-limiting toxicities included diarrhea and sensory neurotoxicity, the latter of which occurred in patients with antecedent neuropathy. The maximum tolerated dose was defined as 1.56 mg/m², and pharmacodynamic studies showed a dose-dependent proteasome inhibition in vivo. One patient with non-small cell lung carcinoma had a partial response (PR) to therapy, whereas 3 other patients had stable disease as their best responses.

Results of another Phase I trial have been reported in patients with advanced hematological malignancies (50). Using a more intensive schedule in which bortezomib was dosed twice weekly for 4 consecutive weeks over a 6-week cycle, the maximum tolerated dose was 1.04 mg/m². Dose-limiting toxicities included thrombocytopenia, hyponatremia, hypokalemia, fatigue, and malaise, whereas peripheral neuropathy was seen in 5 patients, all of whom had received neurotoxic agents previously. Bortezomib again induced a dose- and time-dependent inhibition of proteasome function. Evidence of antitumor efficacy was seen in 9 out of 9 evaluable patients with plasma cell dyscrasias, including 1 patient with multiple myeloma who had a durable complete response (CR). Partial responses were also seen in 2 patients with non-Hodgkin’s lymphoma, including 1 with mantle cell lymphoma, and another with low-grade follicular B-cell lymphoma.

Phase II Studies with Single-Agent Bortezomib. Encouraging preclinical and Phase I studies in plasma cell dyscrasias led to a Phase II trial evaluating bortezomib in patients with multiple myeloma (51). Heavily pretreated patients (202) received 1.30 mg/m² of bortezomib on days 1, 4, 8, and 11 of a 3-week cycle for up to eight cycles. Complete responses, measured by the stringent Bladé criteria (52), were seen in 4% of
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Some inhibitor-mediated apoptosis, and studies in Hodgkin’s disease, mantle cell lymphoma, and chronic myeloid leukemia are also underway. Single-agent bortezomib is being evaluated in a variety of solid tumors, including hepatocellular carcinoma, melanoma, metastatic breast carcinoma, metastatic colorectal carcinoma, metastatic renal cell carcinoma, neuroendocrine tumors, ovarian and primary peritoneal carcinoma, non-small cell lung carcinoma, and sarcoma.

**Bortezomib-Based Combination Therapy.** The ability of proteasome inhibition to enhance chemotherapeutic and radiosensitivity, and overcome drug resistance in preclinical models, has engendered great excitement about the potential of combination regimens with standard chemotherapeutics. To explore this possibility further, a number of Phase I studies evaluating bortezomib in combination with standard chemotherapeutic agents have now been conducted or are currently underway. For example, a clinical trial of the combination of rituximab and bortezomib has been completed recently that defined the dose-limiting toxicities and the dose for additional testing of this combination (55). Likewise, a Phase I trial of the combination of pegylated liposomal doxorubicin (Doxil) and bortezomib in patients with refractory solid tumors is currently ongoing at our institution. Twenty patients with refractory metastatic solid tumors have been treated thus far with bortezomib, which is administered on days 1, 4, 8, and 11 of a 3-week cycle, and pegylated liposomal doxorubicin at 30 mg/m², which is administered on day 4. Doses up to 1.50 mg/m² of bortezomib in this combination have been administered, with low-grade fatigue, nausea, and thrombocytopenia having been the most frequent toxicities. Accrual is ongoing to define a dose for additional testing. These and other trials focusing on a general population of solid tumor patients, or those with hematological malignancies, are summarized in Table 3. On the basis of these study results, it appears that the toxicities of bortezomib in combina-

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Table 3  Phase I combination studies with bortezomib

<table>
<thead>
<tr>
<th>Standard agent</th>
<th>Population</th>
<th>DLTs</th>
<th>MTD (mg/m²)</th>
<th>Lead PI/Institution</th>
<th>Last Update</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irinotecan</td>
<td>Solid tumors</td>
<td>Report pending</td>
<td>Report pending</td>
<td>Ryan</td>
<td>ASCO 2002</td>
</tr>
<tr>
<td>5-Fluorouracil/leucovorin</td>
<td>Solid tumors</td>
<td>Diarrhea</td>
<td>Bortezomib 1.00 5-fluorouracil 500 Leucovorin 20</td>
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<td>Hematologic malignancies</td>
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*DLT, dose-limiting toxicity; MTD, maximum tolerated dose; PI, principal investigator; ASCO, American Society of Clinical Oncology.*
Combination regimens based on bortezomib are also being studied in patients with hematological malignancies. The Phase II multiple myeloma study was designed to allow addition of dexamethasone to patients with progressive disease after two cycles or stable disease after four. A recent analysis of these data indicated that dexamethasone appeared to have an additive effect on response rate, albeit with some increased toxicity (57). Another interesting combination being evaluated in myeloma patients is that of thalidomide and bortezomib. Early results from that Phase I study have shown tolerable toxicity with interestingly enough, no significant increase in neurotoxicity. Responses have even been noted in patients with deletions of chromosome 13, a marker of chemoresistance and poor patient prognosis (58). In our study of the combination of pegylated, liposomal doxorubicin and bortezomib for patients with relapsed and refractory hematological malignancies (59), toxicities have been manageable, and of the first 20 myeloma patients evaluated 5 have achieved a CR, with 2 near-CRs. Moreover, several of these patients, including 2 of those with a CR, had received anthracycline-based regimens previously and either progressed or their disease did not respond to therapy. These results support the hypothesis that bortezomib can increase the sensitivity of transformed cells to standard chemotherapy and possibly enhance their antitumor efficacy.

Conclusions

The UPP impacts on a number of cellular processes crucial to oncogenesis. Proteasome inhibitors are able to induce apoptosis and tumor regression as single agents in a broad spectrum of tumor cell lines, and in in vivo xenograft models. Perhaps more importantly, they have shown the ability to overcome drug resistance and to synergize with a number of conventional therapies. Whereas down-regulation of NFκB activity likely plays an important role in their observed effects, a number of other factors have also been shown to be involved. The selectivity of these agents to cancer cells is intriguing, and may be partly explained by increased proteasome activity and/or increased activity of components of the Ub-conjugating system in transformed cells. Additional investigation into the role that deregulated UPP activity plays in various malignancies, and whether it correlates with tumor responses to these agents, is warranted. These studies may also support the development of drugs that target specific components of the UPP. Phase I and II trials with the proteasome inhibitor bortezomib have demonstrated tolerable toxicities and some clinical responses, with significant activity in multiple myeloma. Data evaluating the efficacy of bortezomib in solid tumors are less mature; however, it is likely that for these diseases proteasome inhibitors will work best as a component of combination therapy. Mature Phase II data evaluating bortezomib in combination with other agents for solid tumors, and trials evaluating its role earlier in multiple myeloma therapy, are eagerly anticipated.

References


