Building on bortezomib: second-generation proteasome inhibitors as anti-cancer therapy

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Inhibition of the proteasome (a highly abundant enzymatic complex responsible for intracellular protein turnover) is an effective anti-cancer therapeutic approach, as demonstrated by the first-in-class agent bortezomib. Various new proteasome inhibitors are now in development, including peptide boronic acid analogs MLN9708 and CEP-18770, peptide epoxyketones carfilzomib and PR-047, and NPI-0052, a β-lactone compound. All are potent inhibitors of proteasome activity in vitro but show differences in enzyme binding kinetics, which might affect their pharmacology and result in different efficacy and safety profiles. Here, we review the second-generation proteasome inhibitors and assess the potential pharmacologic impact of their different chemical properties.

Introduction

Inhibition of the proteasome has emerged as a clinically effective anti-cancer therapeutic approach over the past decade [1]. This has been primarily for hematologic malignancies – including multiple myeloma (MM) [2], non-Hodgkin's lymphoma (NHL) [3], and Waldenström’s macroglobulinemia (WM) – and associated conditions, such as primary systemic amyloidosis. The first-in-class proteasome inhibitor bortezomib (VELCADE®, Millennium Pharmaceuticals, Inc., and Johnson & Johnson Pharmaceutical Research & Development, L.L.C.) has demonstrated the feasibility of this approach and validated the concept of proteasome inhibition [1]. Preclinical studies have suggested that proteasome inhibition with bortezomib results in pleiotropic effects, disrupting multiple cellular signaling pathways and inducing tumor cell death [1,4]. Clinical studies have established substantial activity of bortezomib in various malignancies, as reviewed elsewhere [2,3], and also determined its pharmacokinetics and pharmacodynamics and characterized its safety profile [5].

Some limitations of bortezomib exist, including limited activity in solid tumors [1], emergence of reversible peripheral neuropathy in several patients [6] and the intravenous route of administration. Thus, several new proteasome inhibitors are in development, with the aim of building on the activity seen with bortezomib while improving the safety profile of proteasome inhibition and the convenience of administration. Here, we discuss the key aspects of the development of new proteasome inhibitors, review this second generation of agents and assess the potential pharmacologic impact of the different chemical properties of these agents.

The proteasome as a therapeutic target

The proteasome is a crucial component of the ubiquitin–proteasome system (UPS) [7–9], which is responsible for regulation and degradation of the majority of intracellular proteins. Consequently, its inhibition affects numerous signaling pathways in cells. UPS substrates include proteins responsible for regulating cellular processes such as the cell cycle, growth and proliferation signaling, and pro-apoptotic and anti-apoptotic signaling.

The 26S proteasome comprises a 20S core (Figure 1) containing multiple active enzymatic sites with chymotrypsin-like (β5), trypsin-like (β2), and post-glutamyl peptide hydrolase-like (caspase-like, β1) activities, plus a 19S regulatory cap at either end [10,11]. The 26S proteasome is highly abundant and ubiquitous in cells; for example, Hendil showed that proteasomes represented 0.6% of soluble protein from cultured HeLa human carcinoma cells [12], Tanaka et al. calculated that proteasomes accounted for 1% of total soluble protein in rat liver [13], and Lightcap et al. reported there were $2.7 \times 10^{-19}$ moles of 20S proteasome in a red blood cell and $8 \times 10^{-19}$ moles in a white blood cell, with equivalent proteasome concentrations of 1.9–4.1 and 1–6 μmol/L, respectively [14].
even using the most conservative estimates for calculating the total number of proteasomes in the body or in a patient’s blood and liver, it is clear this number greatly exceeds the number of proteasome inhibitor molecules administered to a patient; for example, there are approximately $5.5 \times 10^{18}$ molecules of bortezomib in a single 3.5 mg vial compared with a total number of proteasomes in human blood and liver of approximately $3.6 \times 10^{18}$ and $3.9 \times 10^{18}$, respectively, or $7.5 \times 10^{18}$ overall, assuming a typical human blood volume of 6 L and liver volume of 1.6 kg (1.6 L). It is also important to note that most cells have a great capacity to synthesize new proteasomes, with the exception of anucleated cells such as red blood cells [15,16].

Compounds that function as proteasome inhibitors bind either reversibly or irreversibly to the active enzyme sites in the 20S proteasome, primarily the chymotrypsin-like site, thus inhibiting their proteolytic function. Five main types of compounds have been identified as proteasome inhibitors, including peptide aldehydes, peptide vinyl sulfones, peptide boronates, peptide epoxyketones (epoxomycin and eponomycin) and \( \beta \)-lactones (lactacystin and derivatives) [10,17]. Only a few compounds have progressed to clinical development, however, with others deemed unsuitable owing to metabolic instability, potency issues or lack of specificity.

The first-in-class proteasome inhibitor bortezomib is a peptide boronic acid analog that is a reversible inhibitor of primarily the chymotrypsin-like activity of the 20S proteasome [10]. Bortezomib is approved for the treatment of MM and the treatment of mantle cell lymphoma (MCL) after at least one prior therapy [5] and has demonstrated activity in various other malignancies. However, it has some shortcomings as a therapeutic agent. To try to overcome these shortcomings in developing second-generation inhibitors, various molecular and chemical characteristics can be altered compared with bortezomib, including chemical class (the proteasome-inhibiting ‘warhead’), potency of the compound for the various proteasome subunits, binding kinetics and route of administration. This is illustrated in Figure 2, which shows the structures of bortezomib and the second-generation inhibitors, and Table 1, which summarizes key data on each agent.

As shown in Figure 2, two other peptide boronic acid analogs are in development. MLN9708 (Millennium Pharmaceuticals, Inc.), which hydrolyses immediately in plasma to MLN2238 [18], is a reversible inhibitor of the chymotrypsin-like subunit of the 20S proteasome that is distinct from bortezomib in having a substantially shorter dissociation half-life [18] (Table 1). In addition, oral bioavailability has been demonstrated with MLN2238 [18]. CEP-18770 (Cephalon) is a P2 threonine boronic acid [19,20] that is another reversible inhibitor, primarily of the chymotrypsin-like activity of the proteasome [20].

Two compounds in the peptide epoxyketone class are being developed by Proteolix. Carfilzomib (formerly PR-171) is an irreversible inhibitor of the chymotrypsin-like activity of the proteasome [16], and PR-047 is an orally bioavailable analog of carfilzomib, again being an irreversible inhibitor of the \( \beta \)5 subunit [21]. Finally, several natural compounds have been identified as inhibitors of the proteasome, and one, NPI-0052, is currently in clinical development at Nereus Pharmaceuticals. NPI-0052, or salinosporamide A, is a \( \beta \)-lactone compound derived from the marine bacterium Salinospora tropica; like carfilzomib and PR-047, it is also an irreversible inhibitor of the \( \beta \)5 subunit [22–24]. As shown in Table 1, given their low nanomolar IC\text{50} values for the \( \beta \)5 subunit, bortezomib and the second-generation inhibitors all represent very effective inhibitors of proteasome activity.
NPI-0052 also has a low nanomolar IC\textsubscript{50} for the trypsin-like (b2) subunit \cite{25}, although the clinical relevance of this remains to be determined.

**Mechanism of action of proteasome inhibition**

Several putative mechanisms of activity of proteasome inhibition have been determined based upon preclinical studies of bortezomib. Bortezomib induces apoptosis in tumor cells via the intrinsic mitochondrial pathway, the extrinsic death-receptor pathway, and the endoplasmic reticulum stress response pathway \cite{4,26,27}. This activity has been suggested to result from inhibition of the degradation of various regulatory and pro-apoptotic proteins \cite{4}, including: inhibition of nuclear factor (NF)-kB activity through the prevention of degradation of its inhibitor IkB; deregulation of the turnover of cyclins and disruption of cyclin-dependent kinase activity; JNK stabilization and Fas upregulation; stabilization of p53, a tumor suppressor; and a shifting of the pro-apoptotic and anti-apoptotic balance in the Bcl-2 family of proteins. In addition, proteasome inhibition with bortezomib results in unfolded protein response disruption, reactive oxygen species generation and oxidative stress.

Preclinical studies of the second-generation proteasome inhibitors have shown similar pleiotropic effects. CEP-18770 demonstrated inhibition of the NF-kB pathway and apoptosis induction through caspase activation in MM cell lines \cite{20}. Carfilzomib demonstrated similar cellular effects in hematologic cell lines, inducing apoptosis via the extrinsic and intrinsic pathways, associated with accumulation of growth arrest markers such as cyclin B1 and the cyclin-dependent kinase inhibitor p21 and stress response markers such as heat-shock proteins hsp27 and hsp70 \cite{16,28}. Studies of NPI-0052 have shown it to result in caspase-8-mediated apoptosis \cite{25,29}, NF-kB inhibition \cite{30–34}, reductions in cellular FLICE-like inhibitory protein (cFLIP) and other anti-apoptotic proteins \cite{30}, Raf-1 kinase inhibitor induction \cite{35}, death receptor 5 upregulation \cite{34}, poly (ADP-ribose) polymerase (PARP) cleavage \cite{31}, and endoplasmic reticulum stress-mediated apoptosis \cite{36}.

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**FIGURE 2**

Chemical structures of bortezomib and the second-generation proteasome inhibitors.

**TABLE 1**

**Properties of bortezomib and the second-generation proteasome inhibitors**

<table>
<thead>
<tr>
<th>Proteasome inhibitor</th>
<th>IC\textsubscript{50} b2/b1 (nM)</th>
<th>IC\textsubscript{50} NF-kB (nM)</th>
<th>Dissociation t\textsubscript{1/2} (min)</th>
<th>Stage of clinical development</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bortezomib</td>
<td>2.4–7.9/590–4200/24–74 \cite{16,18,25}</td>
<td>36–40 \cite{18,25,39}</td>
<td>110 \cite{18}</td>
<td>Approved for MM and MCL</td>
<td>IV (SC also studied) \cite{63}</td>
</tr>
<tr>
<td>MLN9708 [18]</td>
<td>3.4/3500/31</td>
<td>62</td>
<td>18</td>
<td>Phase I</td>
<td>IV (Oral dosing efficacious \textit{in vivo})</td>
</tr>
<tr>
<td>CEP-18770 \cite{19,20}</td>
<td>3.8/\textgreater 100/\textless 100</td>
<td>NR</td>
<td>NR—slowly reversible</td>
<td>Phase I</td>
<td>IV</td>
</tr>
<tr>
<td>Carfilzomib \cite{16}</td>
<td>6/3600/2400</td>
<td>NR</td>
<td>Irreversible</td>
<td>Phase II</td>
<td>IV</td>
</tr>
<tr>
<td>PR-047 \cite{21}</td>
<td>36/NR/NR</td>
<td>NR</td>
<td>Irreversible</td>
<td>Preclinical</td>
<td>Orally bioavailable</td>
</tr>
<tr>
<td>NPI-0052</td>
<td>3.5/28/430 \cite{25}</td>
<td>13–20 \cite{25,39}</td>
<td>Irreversible</td>
<td>Phase I</td>
<td>IV</td>
</tr>
</tbody>
</table>

Abbreviations: IV, intravenous; MCL, mantle cell lymphoma; MM, multiple myeloma; NR, not reported; SC, subcutaneous.
Recently, RNA interference (RNAi) screening studies using bortezomib have identified major pathways affected by proteasome inhibition that might be primarily responsible for its anti-tumor activity, including stabilization of Myc, inhibition of DNA damage repair and interference with protein translation [37]. These key pathways are probably common to all proteasome inhibitors, although further experiments are required for confirmation.

**Preclinical studies and clinical development of the second-generation inhibitors**

Bortezomib resulted in substantial anti-tumor activity in *in vitro* and *in vivo* studies and, through the multiple pathways affected by proteasome inhibition, demonstrated synergistic activity with various conventional and novel therapeutic agents [10]. Similar findings have been reported from preclinical studies of the second-generation inhibitors, as discussed below, although some preclinical studies have suggested differential effects and differential anti-tumor activity between proteasome inhibitors, including between bortezomib and the second-generation inhibitors [16,18,20,25,28,38,39]. However, only clinical studies will be able to determine whether these differences translate into different efficacy and safety profiles; indeed, as discussed later, certain differences between particular proteasome inhibitors that have been highlighted in reports from preclinical studies might not prove to be of relevance in humans.

**MLN9708 (MLN2238)**

*In vivo* studies have demonstrated the efficacy of MLN2238 in CWR22 human prostate xenograft, WSU diffuse large B-cell lymphoma, PHTX-24C primary colon cancer and PHTX-22L primary lymphoma models [18]. Both intravenous and oral dosing were shown to be efficacious in the CWR22 model [18], suggesting a potential alternative route of administration to the intravenous route used for bortezomib. Interestingly, MLN2238 resulted in strong, sustained bone marrow proteasome inhibition but a weaker and less sustained effect in blood after an acute dose of 14 mg/kg in mice [40]. Associated with this finding, statistically significant tumor growth inhibition was seen in an intratibial MDA-MB-231 human breast cancer mouse model [40]. In addition, in a severe combined immunodeficiency mouse WSU tumor xenograft model, higher proteasome inhibition was seen in both the tumor and the bone marrow than in the blood [41], indicating substantial distribution of MLN2238 into these tissues. MLN9708 is currently being investigated in phase I studies in patients with lymphoma and non-hematologic malignancies.

**CEP-18770**

CEP-18770 has demonstrated anti-tumor activity, including complete regressions and survival benefits, in myeloma mouse xenograft models [20]. Synergistic activity has also been reported in combination with doxorubicin, melphalan and arsenic trioxide in myeloma cell lines and with melphalan in a myeloma mouse model, including a statistically significant reduction in tumor growth compared with melphalan alone [42]. A phase I study of CEP-18770 in patients with advanced solid tumors or NHL is underway.

**Carfilzomib**

*In vitro* studies have shown that exposure to carfilzomib of various myeloma, lymphoma, leukemia and solid tumor cell lines and patient cells resulted in substantial cytotoxic activity [16,28,43]. Activity has been seen in dexamethasone-resistant and melphalan-resistant, but not doxorubicin-resistant, MM cells [28]. Carfilzomib was synergistic with dexamethasone in MM cells [28], with idarubicin and cytarabine in acute myeloid leukemia patient cells [43], and with cyclin-dependent kinase-4 and kinase-6 inhibitors in chemoresistant MM cells [44], as well as with BH3 mimetic AT-101 in MCL cell lines [45]. In human tumor xenograft models of colorectal adenocarcinoma, B-cell lymphoma and Burkitt’s lymphoma, carfilzomib resulted in dose-dependent and schedule-dependent anti-tumor activity [16]. In addition, statistically significant tumor growth reductions were seen with carfilzomib plus docetaxel compared with the individual agents alone in a lung carcinoma model and with carfilzomib and liposomal doxorubicin in a colorectal carcinoma model [46]. *In vivo*, dose-linear exposure of carfilzomib was seen in rats and monkeys [47]; prolonged exposure in these animals seemed well tolerated, with no adverse impact on neurobehavioral function [48]. Two phase I studies of carfilzomib have been conducted in patients with multiple tumor types, and two phase II studies are ongoing in patients with relapsed or relapsed/refractory MM, plus a phase Ib study in combination with lenalidomide and dexamethasone in patients with relapsed MM and a phase Ib/II study in patients with solid tumors.

**PR-047**

Studies in mouse xenograft models of NHL and colorectal cancer demonstrated PR-047 to have equivalent anti-tumor activity to carfilzomib [21]. Absolute oral bioavailability of up to 39% was seen in rodents and dogs; after oral administration of PR-047 in mice, rapid absorption, tissue distribution and proteasome inactivation was reported [21]. Like carfilzomib, PR-047 demonstrated synergistic activity with cyclin-dependent kinase-4 and kinase-6 inhibitors in chemoresistant MM cells [44]. At the time of writing, PR-047 has not yet entered clinical trials.

**NPI-0052**

Preclinical studies have shown NPI-0052 to have similar *in vitro* single-agent activity to the other proteasome inhibitors, including in MM [25,49], Hodgkin’s lymphoma, MCL, and other NHL, WM and leukemia cells [29,33,50,51]. *In vivo*, NPI-0052 has shown prolonged survival in animal models of MM [25] and activity in a mouse model of leukemia [29]. Numerous studies in hematologic malignancies have shown NPI-0052 to have synergistic activity in combination with various agents, including with lenalidomide in a mouse model of MM [52], with histone deacetylase inhibitors in leukemia cells [29,53], with tumor necrosis factor and thalidomide in myeloid leukemia cells [30], with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in B-cell NHL cells [34,51], and with the pan-Bcl-2 inhibitor obatoclax in MCL cells [54]. Similarly, in solid tumor cell lines and animal models, NPI-0052 has resulted in enhanced activity when added to various combinations of SN38, oxaliplatin, 5-FU–leucovorin, and bevacizumab in colorectal cancer cells [32], with TRAIL [55] and with the histone deacetylase inhibitor vorinostat [56] in pancreatic cancer cells and mouse models, with TRAIL in TRAIL-resistant prostate cancer cells.
inhibitors. The curve demonstrates that small differences in potency in vitro and in vivo might not result in substantial pharmacologic differences. As shown in Table 1, there are differences in enzyme kinetics (i.e. the dissociation half-lives) between the various proteasome inhibitors. In in vitro studies, whereas carfilzomib, PR-047 and NPI-0052 result in irreversible covalent adduct binding to the β sub-units of the proteasome, bortezomib and CEP-18770 (Millennium Pharmaceuticals, Inc., unpublished data) are slowly reversible, and MLN2238 is much more rapidly reversible. Based on current preclinical data, MLN2238 is thus differentiated from the other second-generation inhibitors and from bortezomib.

These differences in kinetics, in combination with the huge abundance of proteasomes – as discussed earlier – might result in differences in tissue distribution of the various inhibitors, which might lead to differences in safety and efficacy profiles. For example, with slowly reversible and irreversible inhibitors, a substantial proportion of the molecules might bind to proteasomes in red blood cells, the vascular endothelium and well-perfused organs such as the liver (i.e. the most ‘immediately available’ proteasomes in the proximal compartments). These sites might effectively act as a ‘sink’ for these agents, rapidly binding the molecules and affecting distribution into solid tumors and therefore, potentially, efficacy in humans.

This concept is illustrated in in vivo studies of MLN9708/MLN2238 and bortezomib. With MLN2238, which is much more rapidly reversible than bortezomib, higher proteasome inhibition was seen in both the tumor and bone marrow than in the blood in a mouse W52 diffuse large B-cell lymphoma tumor xenograft model [41]; in addition, higher proteasome inhibition in tumor tissues was seen in this mouse model with MLN2238 than with bortezomib, which is consistent with reported improved anti-tumor activities in mouse tumor models [18]. Furthermore, in a CWR22 prostate xenograft mouse model, maximum tumor proteasome inhibition (E_{max}) was 74.9% with MLN2238 versus 44.8% with bortezomib, and the tumor area under the effect versus time curve (AUE_{0-24}) was 1410 versus 804, respectively [18]. By contrast, the blood AUE_{0-24} was 571 and 1200, respectively [18], demonstrating differential distribution of the agents. In this mouse model, MLN2238 also resulted in stronger, sustained bone marrow proteasome inhibition but with a weaker and less sustained effect in blood compared with bortezomib [40], probably associated with their different binding kinetics, with less of the MLN2238 being ‘captured’ by the immediately available proteasomes in the blood. Similarly, the synergistic effects seen with bortezomib and NPI-0052 [58] might be associated with these agents competing for 20S proteasome binding sites, altering their distribution into tissues [39].

It is also important to note that the difference in kinetics between inhibitors classified as irreversible or slowly reversible might not be apparent in humans, owing to both the abundance of proteasomes and the capacity of nucleated cells to synthesize new proteasomes. As noted above, the ‘sink’ effect of the proteasomes in the blood and in hepatocytes might result in similar distributions of irreversible and slowly reversible inhibitors. Furthermore, the pharmacodynamic profiles of irreversible and slowly reversible inhibitors appear essentially the same in tissue and nucleated cells, with recovery of proteasome activity driven by the synthesis of new proteasomes after treatment with both types of inhibitors [16]. For example, in peripheral blood mononuclear cells, recovery of proteasome activity was seen 48–72 h after NPI-0052 administration [15], 50–100% recovery within 24 h was seen in all tissues examined after carfilzomib administration [16,38], and proteasome activity recovered in all tissues except blood within 24–72 h of PR-047 administration [21]. Similar recovery periods have been seen after proteasome inhibition with bortezomib [16] and CEP-18770 [20]. In addition, recovery from proteasome inhibition in HT-29 colon carcinoma cells treated with either bortezomib or carfilzomib appeared essentially the same, with new proteasome synthesis again being the mechanism of recovery of proteasome activity [38].

FIGURE 3
Impact of difference in in vitro potency on in vivo potency of proteasome inhibitors. The curve demonstrates that small differences in potency in vitro – for example, between 10 nM and 50 nM – have no effect on in vivo IC_{50}. These differences in kinetics, in combination with the huge abundance of proteasomes – as discussed earlier – might result in differences in tissue distribution of the various inhibitors, which might lead to differences in safety and efficacy profiles. For example, with slowly reversible and irreversible inhibitors, a substantial proportion of the molecules might bind to proteasomes in red blood cells, the vascular endothelium and well-perfused organs such as the liver (i.e. the most ‘immediately available’ proteasomes in the proximal compartments). These sites might effectively act as a ‘sink’ for these agents, rapidly binding the molecules and affecting distribution into solid tumors and therefore, potentially, efficacy in humans.
In nucleated cells such as red blood cells, which cannot synthesize new proteasomes and have a half-life of approximately 15–17 weeks [14], a prolonged pharmacodynamic effect is seen with irreversible versus slowly reversible proteasome inhibition [15,16]. For example, with carfilzomib [16], PR-047 [21] and NPI-0052 [15], sustained whole-blood proteasome inhibition is seen, whereas recovery of whole-blood proteasome function is seen 48–72 h after bortezomib and CEP-18770 administration [20]. Similarly, less than 50% recovery of 20S proteasome activity was observed in whole blood one week after carfilzomib administration in rats and mice [16], and NPI-0052 was shown to result in sustained whole-blood proteasome inhibition with substantial recovery only by day 7 [15]. As discussed earlier, the pharmacodynamics of the only rapidly reversible proteasome inhibitor currently in the clinic, MLN9708/MLN2238, seem in preclinical studies to be markedly different from those of bortezomib, with a less sustained effect in whole blood compared with a more sustained effect in the tumor and bone marrow [41], reflecting the different binding kinetics of the agents. For example, blood 20S proteasome activity recovered from approximately 80% inhibition immediately after MLN2238 administration to approximately 30% inhibition within 8 h, whereas in tumor tissues, proteasome inhibition remained approximately 70% at this time.

Differences in specificity for the three enzymatic sites and for different isoforms of the proteasome, as well as possible off-target effects identified in in vitro studies [16,41,59], might result in differences in efficacy and utility in humans between proteasome inhibitors and, possibly, between tumor types; these issues will be addressed through the clinical development of the new proteasome inhibitors. In practice, because of the abundance of proteasomes and the greater potency for β5 versus the other subunits seen with bortezomib, the vast majority of this drug will be tightly bound to this site and, thus, unavailable to inhibit other proteases. Given this, a key aspect in developing new proteasome inhibitors with improved efficacy and safety profiles would seem to be modulating tissue distribution through the tailoring of enzyme kinetics. For example, differences in tissue distribution might result in different incidences of peripheral neuropathy, an important toxicity seen with bortezomib [6]. Some preclinical studies have indicated that neuropathy is a class effect of proteasome inhibition that occurs regardless of the chemical ‘warhead’ employed and is associated with cytoplasmic accumulation of ubiquitinated proteins and neurofilaments in the dorsal root ganglia [60,61], whereas other studies have suggested that specific off-target effects of bortezomib, such as inhibition of non-proteasomal proteases, might be associated with neuropathic pain [59]. Altered tissue distribution might ameliorate this adverse effect of treatment; however, further study of the etiology is required. Again, clinical data from the development of the new proteasome inhibitors will help address this issue.

Concluding remarks
The concept of proteasome inhibition as a therapeutic approach in cancer is now well established, and numerous companies are now developing new proteasome inhibitors with the aim of building upon the success of the first-in-class inhibitor bortezomib [62]. Bortezomib and all these second-generation compounds represent highly effective inhibitors of the proteasome, regardless of the type of agent; improving upon bortezomib, therefore, will require modification of the pharmacology of the proteasome inhibitors, effected through changes in binding kinetics and consequent tissue distribution compared with bortezomib. Such changes might address some of the key issues associated with bortezomib, such as improving the efficacy of proteasome inhibition in solid tumors, and limiting therapy-associated peripheral neuropathy. Extensive clinical investigation of the second-generation inhibitors will be required, however, to determine whether the pharmacologic differences between these agents and bortezomib will result in differences in efficacy and safety in patients.

The recent RNAi screening work with bortezomib mentioned earlier has identified key cellular mechanisms associated with the activity of proteasome inhibition [37]. These findings might prove important in the subsequent development of further improved proteasome inhibitors and in the development of rational combination regimens for specific tumors based on a proteasome inhibitor. In addition, our expanding understanding of the UPS is providing further drug development opportunities by identifying additional targets of relevance in cancer in this important cellular housekeeping system [9].

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