Enhancing activity and overcoming chemoresistance in hematologic malignancies with bortezomib: preclinical mechanistic studies

N. Reddy† & M. S. Czuczman*

Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY, USA

Received 16 June 2009; revised 9 December 2009; accepted 15 December 2009

Background: Proteasome inhibition results in antitumor activity through various mechanisms, including disruption of cell cycle progression and control, induction of apoptosis, and inhibition of proliferation.

Design: This review assesses preclinical data on the ability of bortezomib, the first proteasome inhibitor approved for clinical use, to enhance antitumor activity of other agents and to overcome chemoresistance in hematologic malignancies and discusses mechanisms by which such activity arises.

Results: Bortezomib has been shown to affect multiple cellular pathways and levels of numerous intracellular proteins, including targets of importance in hematologic malignancies. These mechanisms have shown additive or synergistic effects in vitro and in vivo with those of conventional therapeutic and novel targeted agents. Additionally, targets of proteasome inhibition are implicated in resistance or lack of sensitivity to different therapies. Bortezomib in combination with other agents has been shown to overcome resistance to those agents and to resensitize cells to agents to which they were previously unresponsive.

Conclusions: This review indicates the potential utility of proteasome inhibition for substantially enhancing activity of other therapeutic approaches. It explains the mechanisms responsible for the observed clinical activity of bortezomib-based regimens and elucidates novel therapeutic approaches through identification of combinations of agents with complimentary mechanisms of action.

Key words: bortezomib, chemoresistance, hematologic malignancies, nuclear factor (NF)-κB, proteasome

introduction

Bortezomib (VELCADE®, Millennium Pharmaceuticals, Inc., Cambridge, MA, and Johnson & Johnson Pharmaceuticals Research & Development, L.L.C., Raritan, NJ), the first proteasome inhibitor to enter the clinic, is approved for the treatment of patients with multiple myeloma (MM) and patients with mantle cell lymphoma (MCL) following at least one prior therapy. Bortezomib is a specific reversible inhibitor of the chymotryptic-like enzymic activities of the 26S proteasome, a key component of the ubiquitin–proteasome pathway (UPP). The UPP is responsible for the orderly degradation of the majority of eukaryotic intracellular proteins, including those involved in cell cycle regulation, cell growth and proliferation, and survival. Therefore, through proteasome inhibition, bortezomib affects multiple cellular pathways and levels of numerous proteins, including various targets of importance in hematologic malignancies. Through this complex mechanism of activity, bortezomib has demonstrated additive or synergistic activity in combination with conventional and novel targeted anticancer agents in preclinical studies, as well as the ability to overcome chemoresistance mechanisms and to resensitize resistant tumor cells to cytotoxic effects of other agents. Improved understanding of these mechanisms will aid development of novel, highly effective, bortezomib-based combination regimens, some of which are already being clinically investigated.

proteasome inhibition: mechanisms of antitumor activity

As reviewed elsewhere, proteasome inhibition results in antitumor activity through various mechanisms [1, 2], including disruption of cell cycle progression and control, induction of apoptosis, and inhibition of proliferation and angiogenesis. Such activity arises through stabilization and prevention from degradation of various intracellular proteins by the 26S proteasome, disrupting the balance between pro-apoptotic and antiapoptotic/pro-survival signaling in cells and down-regulating other signaling pathways involved in...
growth and survival. These multiple mechanisms of activity may combine synergistically with those of other conventional and targeted agents to produce highly active regimens against various tumors.

Bortezomib induces caspase-mediated apoptosis via three distinct pathways (Figure 1)—the classical intrinsic mitochondrial apoptotic pathway, involving caspase-9 activation, the caspase-8-mediated extrinsic death receptor (DR) pathway, and activation of the endoplasmic reticulum (ER) stress response pathway, involving caspase-2/-4/-12. The importance of each pathway in the response to bortezomib may vary between different malignancies, and additive/synergistic activity in combination regimens may arise from targeting different pathways with different combination agents. Mitsiades et al. [3] reported that bortezomib triggers a dual apoptotic pathway in MM.1S myeloma cells. Activation of the intrinsic mitochondrial apoptotic pathway was seen through release of cytochrome c from the mitochondria and caspase-9 activation, with associated down-regulation of Bcl-2 and cellular inhibitor of apoptosis-2 (cIAP-2). Additionally, the extrinsic pathway was shown to be activated; increases were seen in death-inducing receptors Fas and DR5, enhancing Fas-mediated signaling and caspase-8 activation [3]. Downstream effects of bortezomib-induced apoptosis included activation of c-Jun NH2-terminal kinase (JNK) and increased c-Jun phosphorylation, plus decreased cellular FLICE-like inhibitory protein (c-FLIP), a negative regulator of Fas-induced apoptosis [3]. Gomez-Bougie et al. [4] showed that apoptosis induction by bortezomib in MM cells was also associated with up-regulation of the pro-apoptotic Noxa and cleavage of the antiapoptotic protein Mcl-1. Preclinical studies have also shown proteasome inhibitors to activate the ER stress response in MM, associated with caspase-2/-4/-12 activation and disruption of the unfolded protein response (UPR) [5, 6]. Furthermore, it has been shown that increasing 26S proteasome workload using agents causing additional ER stress results in marked enhancement of cells’ susceptibility to proteasome inhibition [7]. In one study, activation of the ER stress response and proteotoxicity were indicated as mechanisms responsible for bortezomib-induced apoptosis in MM tumor cells and dendritic cells, thereby disrupting their interaction in the tumor microenvironment [8].

Inhibition of nuclear factor (NF)-κB activity has appeared an important downstream target of bortezomib’s mechanism of activity, possibly giving rise to a number of the effects described above [3]. NF-κB is responsible for activating transcription of growth factors, cell adhesion molecules, angiogenesis factors, and antiapoptotic factors such as Bcl-2 (Figure 1) [1]. Ma et al. [9] reported that bortezomib substantially reduced NF-κB DNA-binding activity and nuclear translocation in chemosensitive and chemoresistant MM cell lines. In other studies, bortezomib inhibited paracrine cell growth and signaling in the bone marrow microenvironment, through inhibition of interleukin (IL)-6 secretion [10], while
Roccaro et al. [11] showed that bortezomib abrogated proliferation when MM.1S cells were bound to endothelial cells derived from MM patients, mediating angiogenesis via dose-dependent inhibition of vascular endothelial growth factor and IL-6 secretion by endothelial cells. Hideshima et al. [12] reported bortezomib-induced expression of the tumor suppressor p53 and its positive regulator murine double minute oncogene and phosphorylation of p53, in response to DNA damage, while in another report, Hideshima et al. [10] noted that bortezomib inhibited IL-6-mediated activation of p42/44 mitogen-activated protein kinase (MAPK) in MM.1S cells. These effects may be due to inhibition of NF-κB activity. However, Hideshima et al. [13] recently showed that bortezomib induced NF-κB activation via the canonical pathway in MM cell lines and patient tumor cells, through induction of IκB kinase-β phosphorylation, indicating that its cytotoxicity may not be attributable to inhibition of canonical NF-κB activity in MM cells. Nevertheless, bortezomib may inhibit noncanonical NF-κB activation and inducible NF-κB activity, and Hideshima et al. [13] noted that while NF-κB activation was seen in peripheral blood mononuclear cells, NF-κB inhibition was seen in bone marrow stromal cells, indicating differential NF-κB effects depending on cell type. Together, these findings indicate that inhibition of NF-κB activity may be less important than initially thought in the mechanism of activity of bortezomib.

Similar mechanisms of activity have been reported in B-cell lymphoma cell lines. Activation of caspase-8 and caspase-9 and subsequent apoptosis were seen in DHL-7 and DHL-4 lymphoma cell lines on exposure to bortezomib, together with inhibition of NF-κB DNA-binding activity [14]. In contrast to MM cell lines, increases in the antiapoptotic Bcl-2 were seen in both cell lines, accompanied by increases in the pro-apoptotics Bax and Bak, leading to the subsequent apoptotic cascade [14]. Similarly, in MCL cell lines, bortezomib resulted in inhibition of constitutive NF-κB activity and cyclin D1 expression, leading to rapid apoptosis induction associated with mitochondrial disruption [15]. The antiapoptotics Bcl-XL and Bcl-2 were down-regulated and caspase-3 was activated, mediating Bcl-2 cleavage and mitochondrial cytochrome c release [15]. Perez-Galan et al. [16] demonstrated that bortezomib increased reactive oxygen species (ROS) generation and up-regulated the pro-apoptotic Noxa in MCL cell lines. Noxa counteracted accumulation of the antiapoptotic Mcl-1 seen following bortezomib treatment by binding to it, promoting release of Bak and subsequent caspase activation [16]. Meanwhile, bortezomib and other proteasome inhibitors induced apoptosis in chronic lymphocytic leukemia cells and a Burkitt lymphoma cell line via the DR apoptotic pathway; proteasome inhibitor-induced apoptosis was associated with up-regulation of TRAIL and DR4 and DR5 DRs, plus decreased levels of c-FLIP [17].

**enhanced antitumor activity with bortezomib in combination with other agents**

Bortezomib’s mechanism of activity is highly relevant in various hematologic malignancies. In addition to direct cytotoxic activity, proteasome inhibition has been shown to be additive or synergistic with conventional therapeutic and novel targeted agents. The number of pathways affected by bortezomib treatment (Figure 1) indicates the broad utility of proteasome inhibition as a strategy for enhancing antitumor activity and thus providing the basis for highly active clinical combination regimens.

**multiple myeloma**

Bortezomib has been investigated extensively in myeloma cells in combination with other agents (Table 1). Bortezomib has demonstrated additive activity with dexamethasone [10], enhanced activity with lenalidomide [18], and synergistic activity with melphalan [19]. In addition to synergy with doxorubicin [19], bortezomib has demonstrated synergistic activity with other topoisomerase IIα-targeted agents including etoposide, daunorubicin, and etonafide. Enhanced levels of topoisomerase IIα, which is regulated by proteasomal degradation, were seen following bortezomib exposure in RPMI8226/S and 8226Dox1V cell lines; consequently, sequence-dependent synergistic cytotoxicity was seen with bortezomib followed by etonafide [27].

Joint targeting of the proteasome with bortezomib and histone deacetylase (HDAC) inhibition has demonstrated synergistic activity in myeloma cell lines, indicating a potential therapeutic approach [20–22]. This activity appears to arise via an enhanced ER stress response (Figure 1), with bortezomib plus HDAC inhibition resulting in enhanced disruption of the UPR–aggresome pathway [20], as well as through the mitochondrial apoptotic pathway via an enhanced ROS-dependent mechanism [21]. This was associated with cytochrome c and second mitochondria-derived activator of caspasas (Smac)/Diablo mitochondrial release and down-regulation of the antiapoptotic proteins Mcl-1 and X-linked inhibitor of apoptosis protein (XIAP) [21]. Targeting both proteasome and aggresome pathways induces cell stress via accumulation of polyubiquitinated proteins and therefore activates the apoptotic cascade. HDAC6 blocks the aggresome and in combination with bortezomib synergistically induces cytotoxicity in myeloma cell lines [30].

Farnesyltransferase inhibition is another targeted approach that has shown additive/synergistic activity with bortezomib in myeloma cell lines [6, 23, 24], again indicating a potential combinatorial therapeutic approach. An enhanced ER stress response was noted with bortezomib plus tipifarnib, which resulted in the combination overcoming cell adhesion-mediated drug resistance (CAM-DR) [6]. Enhanced intrinsic and extrinsic apoptotic pathways were seen with the combined mechanisms of activity, associated with a reduction in p-Akt level, indicating reduced PI3K/Akt signaling [23, 24].

Combination of proteasome inhibition and p38 MAPK inhibition has also demonstrated augmented activity in myeloma cells [25, 26]. Inhibiting p38 MAPK abrogates the heat-shock protein response that is a component of the stress response to bortezomib, via inhibition of heat-shock protein 27 (Hsp27), as illustrated in Figure 1, thereby optimizing cytotoxic activity of bortezomib alone or in combination with the Hsp90 inhibitor 17-AAG [25, 26]. Furthermore, p38 inhibition also up-regulates p53 and down-regulates the antiapoptotics Bcl-X(L) and Mcl-1 [25].
Additive/synergistic activity has been reported with bortezomib plus conventional or novel agents in various lymphoma subtypes, particularly MCL (Table 2). Sequence-dependent synergistic inhibition of proliferation and apoptosis induction was seen in MCL cell lines with bortezomib plus cytarabine, mitoxantrone, fludarabine, or gemcitabine [31]. Furthermore, bortezomib was synergistic with rituximab plus...
cyclophosphamide in various MCL cell lines and patient cells, and the combination eradicated Jeko-1 s.c. tumors in a severe combined immunodeficiency (SCID) mouse model [33]. As in MM studies, reported mechanisms resulting in these synergistic effects included enhancement of caspase-mediated apoptosis, caused by targeting of different but interlinked signaling pathways of relevance in MCL/lymphoma cells, as illustrated in Figure 1. Bortezomib and the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) demonstrated synergistic cytotoxic effects in MCL cell lines associated with markedly increased ROS generation, caspase-3, -8, and -9 activation, and decreased NF-kB activity [34], while significantly increased apoptosis was also seen with bortezomib plus LBH589 in MCL cells, associated with higher levels of the pro-apoptotics C/EBP-homologous protein and Noxa and an enhanced ER stress response [35].

Table 2. Key preclinical studies of bortezomib in combination with conventional and novel agents in MCL and other lymphomas demonstrating additive or synergistic activity

<table>
<thead>
<tr>
<th>Combination agents</th>
<th>Enhanced activity reported</th>
<th>Proposed mechanisms of additive/synergistic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytarabine [31]</td>
<td>Synergistic inhibition of proliferation and induction of apoptosis with cytarabine followed by bortezomib</td>
<td>Further enhancement of cytarabine-induced pro-apoptotic proteins</td>
</tr>
<tr>
<td>Rituximab [32]</td>
<td>Synergistic apoptosis in Jeko, Mino, and SP53 cell lines and patient samples</td>
<td>Caspase-dependent and -independent pathways, reduced NF-kB activity, synergistic induction of the pro-apoptotic Bax, and elimination of the antiapoptotic Bcl-2</td>
</tr>
<tr>
<td>Rituximab + cyclophosphamide [33]</td>
<td>Synergistic growth inhibition and significantly enhanced apoptosis in SP53, Mino, Granta 519, and Jeko-1 MCL cells; eradication of Jeko-1 s.c. tumors in a SCID mouse model</td>
<td>Activation of caspase-3, -8, and -9 and PARP</td>
</tr>
<tr>
<td>HDAC inhibitors</td>
<td>SAHA [34]</td>
<td>Synergistic cytotoxicity in Jeko-1, Granta-519, and Hbl-2 cells</td>
</tr>
<tr>
<td></td>
<td>LBH589 [35]</td>
<td>Significantly increased apoptosis in MCL cells</td>
</tr>
<tr>
<td></td>
<td>Romidepsin and belinostat [36]</td>
<td>Synergistic induction of cell death in JVM-3 and MEC-2 CLL cells</td>
</tr>
<tr>
<td>Bcl-2 family inhibitors</td>
<td>Bcl-2 antisense oblimersen + cyclophosphamide [37]</td>
<td>At least additive activity in Rl and SKI-DLCL-1 lymphoma cells, complete disease regression in SCID mouse model of SKI-DLCL-1</td>
</tr>
<tr>
<td></td>
<td>Pan-Bcl-2 inhibitor GX15-070 [38]</td>
<td>Synergistic antitumor activity in rituximab-sensitive and rituximab-resistant lymphoma cell lines and in treatment-refractory primary DLBCL patient cells</td>
</tr>
<tr>
<td></td>
<td>Pan-Bcl-2 inhibitor GX15-070 [39]</td>
<td>Synergistic cytotoxicity in UPN-1, Jeko, and Granta MCL cell lines and primary patient cells</td>
</tr>
<tr>
<td></td>
<td>Bcl-2 antagonist HA14-1 [40]</td>
<td>Synergistic induction of apoptosis in germinal-centered and ABC DLBCL cells</td>
</tr>
<tr>
<td>Other agents</td>
<td>Akt inhibitor perifosine [41]</td>
<td>Synergistic activity in the WM cell lines BCWM.1 and WM-WSU</td>
</tr>
<tr>
<td></td>
<td>Protein kinase C inhibitor enzastaurin [42]</td>
<td>Synergistic activity in the WM cell lines BCWM.1 and WM-WSU</td>
</tr>
</tbody>
</table>

MCL, mantle cell lymphoma; NF, nuclear factor; PARP, poly ADP ribose polymerase; SCID, severe combined immunodeficiency; HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid; ROS, reactive oxygen species; CHOP, C/EBP-homologous protein; ER, endoplasmic reticulum; CLL, chronic lymphocytic leukemia; XIAP, X-linked inhibitor of apoptosis protein; DLBCL, diffuse large B-cell lymphoma; JNK, c-Jun NH2-terminal kinase; ABC, activated B-cell; WM, Waldenström’s macroglobulinemia; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase.
Bortezomib plus the pan-Bcl-2 inhibitor GX15-070 resulted in enhanced activation of the mitochondrial apoptotic pathway via the mechanisms illustrated in Figure 1; GX15-070 abrogated bortezomib-induced accumulation of the antiapoptotic Mcl-1 and enhanced up-regulation of the pro-apoptotics Noxa, Bak, and Bax, resulting in loss of mitochondrial membrane potential, and caspase-3 activation [39]. Inhibition of the antiapoptotic Bcl-2 in combination with proteasome inhibition has demonstrated enhanced activity in other lymphoma cell lines, indicating a potential therapeutic approach. O’Connor et al. showed that bortezomib, oblimersen (antisense Bcl-2), and cyclophosphamide had additive activity in lymphoma cells, markedly improving tumor cell death. In vivo, this triplet resulted in pathological complete regression of disease in a SCID mouse model [37]. As in MCL, bortezomib plus GX15-070 demonstrated synergistic apoptotic and antiproliferative activity in both rituximab-sensitive and rituximab-resistant lymphoma cell lines through up-regulation of Puma and Noxa, an effect also seen in ‘treatment-refractory’ primary diffuse large B-cell lymphoma (DLBCL) patient cells [38]. Similarly, the small-molecule Bcl-2 antagonist HA14-1 resulted in synergistic apoptosis in both germinal-centered and activated B-cell (ABC)-type DLBCL cells, associated with Bax/Bak translocation, cytochrome c mitochondrial release, and caspase activation; JNK activation was increased and ER stress was induced, as evidenced by caspase-2 and -4 activation [40].

The dual apoptotic pathways have been shown to be important for the synergistic effects observed with other combinations in Waldenström’s macroglobulinemia (WM) cell lines. Combined inhibition of the pro-survival P13K/Akt- and extracellular signal-regulated kinases-signaling pathways with perifosine likely contributed to synergistic activity seen with this agent and bortezomib in the BCWM.1 and WM-WSU cell lines [41]. Synergistic activity reported with the protein kinase C inhibitor enzastaurin and bortezomib in the same cell lines may also have been due to inhibition of Akt signaling, associated with enhanced caspase-3, -8, and -9 activation [42]. These findings provide the rationale for bortezomib-based combination therapy approaches in WM with the aim of enhancing antitumor effects through targeting multiple pathways of relevance.

**overcoming resistance to other agents with bortezomib alone and in combination**

Targeting cellular mechanisms and signaling pathways involved in the resistance of different malignancies with bortezomib may restore sensitivity and overcome resistance and re sensitize cells to agents to which they were not responsive.

**multiple myeloma**

Bortezomib has been shown to overcome dexamethasone resistance in myeloma cells [10, 43]. IL-6 is an inhibitor of dexamethasone-induced apoptosis, and so the inhibitory effect of bortezomib on IL-6, via NF-κB inhibition, may explain how dexamethasone resistance is overcome [10]. Elevated expression of Hsp27 also confers dexamethasone resistance in myeloma cell lines through inhibition of mitochondrial release of cytochrome c and Smac [44]. Bortezomib induced apoptosis in dexamethasone-resistant cells through the disruption of Hsp27–actin complexes, and addition of Hsp27 antisense to bortezomib to further target this resistance mechanism resulted in enhanced antmyeloma activity [43]. Bortezomib and the HDAC inhibitor LBH589 also demonstrated synergy against the MM.1R cell line via activation of the UPR–aggresome pathway [20], and bortezomib plus sodium butyrate or SAHA showed synergy in myeloma cell lines resistant to dexamethasone or doxorubicin through enhanced activation of the mitochondrial apoptotic pathway [21]. These studies indicate the clinical potential of novel bortezomib-based regimens, particularly in relapsed/refractory disease.

Bortezomib has also demonstrated single-agent activity in the doxorubicin-, mitoxantrone-, and melphalan-resistant myeloma cell lines [9, 10] through inhibition of NF-κB, over-activity of which may be associated with chemoresistance [9]. Bortezomib also substantially enhanced sensitivity to these agents in combination in chemoresistant cell lines [9, 19]. Mitsiades et al. [19] indicated that these effects of bortezomib were due to down-regulation of the antiapoptotic proteins Bcl-2, A1, cIAP-2, XIAP, and FLIP, via inhibition of NF-κB activation, as well as inhibition of genotoxic stress response pathways, the latter mechanism restoring sensitivity of myeloma cells to DNA-damaging chemotherapy agents. They also demonstrated that bortezomib overcame CAM-DR [19] through lowering of FLIP expression and activating the Fas-dependent caspase-8 apoptotic pathway [3]. Bortezomib treatment also overcame anthracycline chemoresistance in the topoisoasemase II-deficient 8226/Dox1V myeloma cell line. Sensitivity to etohonafide was enhanced (mean 21% decrease in IC_{50}) through bortezomib-induced up-regulation of topoisoasemase IIz, which is regulated via proteasome degradation [27].

**lymphoma**

An increase in NF-κB activity has been associated with development of resistance to rituximab in non-Hodgkin’s lymphoma (NHL) cell lines. Olejniczak et al. [45] demonstrated decreased levels of IkB and increased levels of NF-κB in rituximab-resistant cells compared with parental cells. Increased levels of the antiapoptotic proteins survivin and Bcl-2 were also noted, likely due to increased NF-κB transcriptional activity, enhancing rituximab resistance [45]. In addition, Jazirehi et al. [46] showed that rituximab-resistant clones of the Ramos and Daudi B-cell NHL cell lines displayed constitutive hyperactivation of the NF-κB pathway, which resulted in overexpression of the antiapoptotic proteins Bcl-2, Bcl-X(L), and Mcl-1. They demonstrated that both rituximab-sensitive and rituximab-resistant clones of these cell lines were resistant to cytotox drugs; however, pretreatment of the cells with bortezomib sensitized them to chemotherapy and synergistic activity was seen, likely due to inhibition of NF-κB activity by bortezomib [46]. Recently, it has been reported that the addition of bortezomib to chemotherapy demonstrates synergistic activity leading to higher response rates in patients with ABC-type DLBCL, which is characterized by constitutive NF-κB activation [47].


overcoming resistance to bortezomib

Just as bortezomib treatment may overcome resistance or sensitize tumor cells to the cytotoxic effects of agents through its effects on cellular pathways of relevance, resistance or refractoriness to bortezomib may be overcome by targeting specific pathways associated with bortezomib resistance with alternative agents. For example, cells up-regulate heat-shock proteins in response to cellular stress and this may render bortezomib less effective. Indeed, overexpression of Hsp27 has been shown to confer bortezomib resistance in lymphoma cell lines [48], possibly through inhibition of bortezomib-induced mitochondrial apoptotic signaling, as has overexpression of Hsp70 and Hsp90. Synergistic activity has been reported between bortezomib and the Hsp90 inhibitor 17-AAG in a bortezomib-resistant MM cell line and patient cells [49]. Combination with 17-AAG inhibited the Hsp90 stress response, and 17-AAG counteracted bortezomib resistance by further impairing the ability of cells to withstand ER stress generated by proteasome inhibition; the combination resulted in enhanced caspase-12 activation [49]. Similarly, Hsp27-mediated resistance may be overcome through use of bortezomib in combination with antisense Hsp27; in bortezomib-resistant DHL-4 cells, which overexpress Hsp27, Chauhan et al. [48] showed this combination to produce an apoptotic response, whereas ectopic expression of Hsp27 in DHL-6 cells, which do not overexpress Hsp27 and are sensitive to bortezomib, rendered these cells resistant to bortezomib-induced apoptosis.

Attenuation of bortezomib-mediated induction of the heat-shock protein and stress response pathways has also been demonstrated through the strategy of IL-6 inhibition [50]. Bortezomib in combination with CNT0328, an IL-6-neutralizing antibody, resulted in additive-to-synergistic antitumor activity in IL-6-dependent myeloma cell lines KAS-6/1 and ANBL-6 and the IL-6-independent cell lines H-929, MM.1S, and RPMI8226 [50]. CNT0328 inhibited IL-6 signaling and attenuated bortezomib-induced up-regulation of Hsp70 and Mcl-1. In addition, ABT-737, an inhibitor of antiapoptotic proteins Bcl-2, Bcl-X(L), and Bcl-w, demonstrated activity in bortezomib-refractory patient myeloma cells [28], potentially by overcoming Bcl-2-mediated resistance.

Recent studies have indicated that bortezomib resistance in a number of MM [51] and MCL [52] cell lines may be due to the presence of proteasome inhibition-resistant pathways of constitutively NF-κB activity; the combination of bortezomib with perillyl alcohol, a known suppressor of proteasome inhibition-resistant pathways of NF-κB activity, resulted in synergistic cytotoxicity in Rec-1 MCL cells that were resistant to the cytotoxic effects of bortezomib alone [52].

discussion

As our understanding of the molecular characteristics of various hematologic malignancies has improved, novel therapies targeted at specific pathways of relevance have been investigated extensively in preclinical studies and shown to offer notable activity either alone or in combination with other agents. As reviewed here, bortezomib has been shown to be a highly effective combination agent, sensitizing tumor cells to conventional and novel therapies and offering synergistic activity through complementary mechanisms of cytotoxicity with other agents. These findings have provided the rationale for clinical investigation of bortezomib-based combination regimens.

Bortezomib has been studied in the clinical setting in combination with conventional and novel therapeutic agents, notably in front-line and relapsed MM. Substantial activity has been reported from studies in newly diagnosed MM of bortezomib in combinations incorporating melphalan, doxorubicin or pegylated liposomal doxorubicin, dexamethasone, and lenalidomide/thalidomide, perhaps reflecting the marked synergistic or additive activity seen in preclinical studies. Notable activity has also been reported with combinations involving these agents in patients with relapsed and/or refractory MM, as reviewed by Richardson et al. [53]. Interestingly, bortezomib-based combination regimens have demonstrated activity in patients in whom treatment with one or more of the agents in the combination had failed previously [53], indicating that the addition of bortezomib may re-sensitize patients to these agents. Furthermore, reflecting the combinations with novel targeted agents that demonstrated enhanced apoptosis through synergistic mechanisms of action, bortezomib is being investigated in studies in patients with relapsed/refractory MM and/or lymphoma in combination with Hsp90 inhibitor 17-AAG (NCT00546780, NCT00514371), HDAC inhibitors SAHA (e.g. NCT00773747, NCT00773838, NCT00703664), LBH589 (NCT00532389) and romidepsin (NCT00765102, NCT00431990), p38 MAPK inhibitor SCIO-469 (NCT00087867, NCT00095680), and Akt inhibitor perifosine (NCT00401011), with encouraging early activity having been reported. In addition, bortezomib is being investigated with rituximab in a phase III study in patients with relapsed NHL (NCT00312845), while bortezomib plus Bcl-2 family inhibitor GX15-070 is being studied in early-phase trials in patients with relapsed/refractory MCL (NCT00407303), lymphoma (NCT00538187), and MM (NCT00719901).

Promising clinical data, together with the findings from preclinical studies, indicate that bortezomib-based combinations may have an important role in the future in the treatment of MM and other B-cell hematologic malignancies, offering enhanced activity compared with individual agents or regimens alone. Importantly, these regimens offer the possibility of overcoming chemoresistance, thereby potentially enabling continued use of drugs to which patients have become refractory, as well as sensitization of tumors to agents to which they would otherwise effect a pro-survival response. Ultimately, given the complexity and range of different subtypes of hematologic malignancies, the emergence of combination therapies, particularly with novel targeted agents, may offer the potential for individualized therapy, targeted at the specific pathways of importance in an individual’s malignancy. Extensive clinical research is ongoing and additional translational research will be required to further validate the preclinical data reviewed here, notably regarding combinations of bortezomib with novel targeted agents and the optimal sequencing of agents in combination regimens. Never proteasome inhibitors that block the enzymic activity of the
proteasome, specifically caspases, are under preclinical and clinical investigation.

funding
Millennium Pharmaceuticals, Inc.

acknowledgements
The authors would also like to thank Steve Hill and Jane Saunders for editorial assistance in the development of this manuscript. Steve Hill is a medical writer and Jane Saunders is a medical editor with FireKite. NR and MSC were fully responsible for the content and all editorial decisions for this manuscript.

references


