Combination Therapy of Bortezomib with Novel Targeted Agents: An Emerging Treatment Strategy

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Abstract

Clinical trials evaluating combinations of targeted agents with bortezomib, the first-in-class proteasome inhibitor, have been initiated, with the objective of enhancing its single agent activity in hematologic malignancies (myeloma, mantle cell lymphoma), as well as expanding its efficacy in solid tumors. In most cases, preclinical studies have provided a supportive rationale for designing these doublet combination studies. Novel, small molecule–targeted agents being investigated with bortezomib in clinical trials include protein deacetylase inhibitors, kinase inhibitors, farnesyltransferase inhibitors, heat-shock protein 90 inhibitors, pan-Bcl-2 family inhibitors, and other classes of targeted inhibitors. Preliminary clinical data, available from a number of ongoing trials, suggest that most of these combinations are well tolerated and some have promising clinical efficacy that will require subsequent confirmation. Translational studies, conducted as part of the trials, may provide important insights into the putative mechanism of action delineated by preclinical studies of the combinations. The emergence of novel proteasome inhibitors may also expand the opportunities for optimizing these combination therapies. There is potential for an increasingly broad clinical trials program to investigate this therapeutic approach in a range of tumor types, as well as to consider additional agents in sequence or in combination.

The complex and heterogeneous array of genomic and epigenetic alterations that has been identified in many tumors, and is associated with a multitude of processes (proliferation, metastasis, chemoresistance) critical for cancer progression, has shaped an emerging consensus that combination strategies employing multiple targeted agents warrant evaluation as an important therapeutic strategy. Numerous small molecule–targeted therapies with independent mechanisms of action that inhibit key cellular proteins or signaling pathways in various cancers are currently in development. Doublet combinations of targeted therapies are being assessed in a variety of tumors, usually testing two agents that have different targets, nonoverlapping toxicity, and some rationale for evaluation (preclinical activity or clinical efficacy).

This review focuses on the clinical development program assessing combinations of targeted agents with the first-in-class proteasome inhibitor bortezomib (VELCADE, Millennium Pharmaceuticals, Inc. and Johnson & Johnson Pharmaceuticals Research & Development, L.L.C.). Bortezomib has been approved by the U.S. Food and Drug Administration (FDA) for multiple myeloma (MM; refs. 1–3), and recently for relapsed mantle cell lymphoma (MCL; ref. 4). It has generally been ineffective as monotherapy for the treatment of a wide variety of solid tumors. Bortezomib, through inhibition of the 26S proteasome and subsequent effect on multiple key cellular pathways, has shown increased and/or synergistic activity with several novel targeted agents, indicating its potential to substantially enhance the clinical activity of these novel therapies. Most of the clinical trials of these combinations were initiated as a result of relevant in vitro studies, which have provided a supportive scientific rationale. An increase in the number of these studies is expected in coming years, on the basis of emerging data with new agents, which is expanding our understanding of the molecular pathways important in cancer progression.

Bortezomib

Bortezomib is a dipeptidyl boronic acid analog that reversibly inhibits the 26S proteasome by binding to N-terminal threonine residues in the active site of the chymotrypsin-like catalytic region (5). As reviewed elsewhere, proteasome inhibition results in anticancer activity through several different mechanisms, including disruption of cell cycle progression, induction of apoptosis, inhibition of proliferation, and anti-angiogenesis (6). The key proteins and signaling pathways affected, and subsequent
Translational Relevance

The expanding early-phase clinical trial program of a broad range of bortezomib-based combinations with novel targeted agents, including histone deacetylase inhibitors, kinase inhibitors, farnesyltransferase inhibitors, heat-shock protein 90 inhibitors, and the pan-Bcl-2 family inhibitors, is reviewed. Early clinical data may identify new therapeutic strategies and provide an opportunity for an increasingly broad clinical trials program to investigate this therapeutic approach in a range of tumor types, particularly hematologic malignancies.

Ongoing translational research will help validate preclinical findings and improve our understanding of the molecular mechanisms of various cancer types, the relevance of pathways to cancer survival, and the molecular and/or genetic markers associated with response and/or outcome with bortezomib and novel targeted therapies. Such information may allow development of combination regimens optimized for specific tumor subtypes, providing tailored therapy for individual patients on the basis of the molecular and/or genetic characteristics of their disease.

cellular effects, are summarized in Fig. 1. Bortezomib is preclinically active in many hematologic and solid malignancies as a single agent and enhances the activity of many chemotherapy agents in models of MM, acute leukemia, and lung and pancreatic cancers (6).

Bortezomib can overcome or reverse chemoresistance and increase sensitivity to specific agents, including melphalan, doxorubicin, mitoxantrene, and dexamethasone (7). Such enhanced activity in combination has translated into substantial clinical activity in large clinical trials; for example, bortezomib plus liposomal doxorubicin has been approved in relapsed MM (8), and on the basis of the pivotal VISTA trial with bortezomib, melphalan, and prednisone in newly diagnosed MM, bortezomib has recently been approved for the treatment of newly diagnosed MM patients (9). These findings indicate the broad value of proteasome inhibition as a strategy for substantially increasing antitumor activity.

Bortezomib in Combination with Novel Targeted Therapies

Combinations of bortezomib and novel targeted therapies may act synergistically to increase antitumor activity and overcome specific cellular resistance and/or anti-apoptotic mechanisms. Preclinical data on combinations of bortezomib and novel targeted therapies currently being investigated are summarized in Supplementary Table S1, together with possible mechanisms responsible for the enhanced activity of each combination. As yet, few resistant models have been tested with the various novel combinations (10). Table 1 provides an overview, and Supplementary Table S2 provides a detailed summary, of the ongoing early-phase clinical trial program of these combination regimens, which are reviewed below.

Histone deacetylase inhibitors

Histone deacetylase inhibitors (HDACi) are a class of cancer therapeutic agents that regulate gene expression by globally increasing histone acetylation (11). The antitumor activity of HDACi involves multiple mechanisms, including transcriptional upregulation of genes involved in apoptosis, cell cycle control, DNA repair, and differentiation (12). HDACi also induce acetylation of nonhistone proteins, which may contribute to antitumor activity (13). A structurally diverse group of compounds with varying specificity against the spectrum of identified histone deacetylases has been identified, and preclinical studies indicate that these agents have potent anticancer activities (11). One HDACi, vorinostat, has been approved by the FDA for the treatment of cutaneous T-cell lymphoma (14). Clinical trials are currently evaluating the combination of bortezomib with at least four HDACi: vorinostat, belinostat, panobinostat, and romidepsin (Table 2).

Preclinical assessments of combinations of bortezomib and HDACi have identified several potential mechanisms resulting in synergistic antitumor activity:

1. Nuclear factor-κB (NF-κB) effects: Bortezomib blocks IκBα degradation, inhibiting NF-κB activation and diminishing expression of NF-κB target genes. These effects enhance the antitumor lethality of HDACi as manifested by enhanced HDACi-mediated reactive oxygen species and apoptosis in MM (15), non-small cell lung carcinoma (NSCLC; ref. 16), chronic lymphocytic leukemia (17), MCL (18) cell lines, and imatinib-resistant leukemia cells (19).

2. Aggresome disruption: Aggresome formation, dependent on HDAC 6, is induced as a cytoprotective response to the increasing burden of misfolded proteins created by bortezomib treatment of cancer cells. Inhibition of HDAC 6 activity by HDACi, like vorinostat or tubacin leads to aggresome disruption and inactivation with subsequent onset of apoptosis in pancreatic cancer cells (20) and MM cells (21).

3. Endoplasmic reticulum stress response: The unfolded protein response, an evolutionarily conserved adaptive response, is overwhelmed in response to the combined insults of bortezomib (increased unfolded proteins, ref. 22; NF-κB inactivation) and HDACi. This response triggers events leading to a striking increase in mitochondrial injury, caspase activation, and apoptosis, in some cases characterized by increased production of Bcl-Xs accompanied by activation of Bid and production of caspase-8 and -3 (23). The combination of bortezomib and vorinostat has been the most extensively studied. A phase III trial
of this doublet is evaluating a conventional bortezomib dose and schedule with 400 mg of vorinostat on days 1 through 14 of a 21-day cycle in patients with relapsed and/or refractory MM (one to two prior treatments). This definitive study was initiated after two phase I dose-escalation trials (24, 25) of bortezomib-vorinostat in relapsed and/or refractory MM (NCT00310024 and NCT00111813) established that vorinostat and bortezomib were well tolerated (nausea, diarrhea, thrombocytopenia most common toxicities), and 18 out of 55 evaluable patients (33%) achieved a very good partial response (VGPR) or partial response (PR). Approximately 40% of patients with prior bortezomib therapy also had a response (24). In total, five phase I dose-escalation studies of bortezomib-vorinostat in MM or solid tumor patients, assessing a variety of administration schedules for both agents, are ongoing or completed. In a phase I study of patients with unresectable or metastatic solid tumors (NCT00227513), objective responses were noted in two patients, one with a soft tissue sarcoma (PR lasting >9 months) and an NSCLC patient. Because this study suggested that the bortezomib-vorinostat combination may be efficacious in solid tumors, a number of phase II trials are evaluating regimen in NSCLC (NCT00798720), glioma (NCT00641706), and non-Hodgkin’s lymphomas (NHL; NCT00703664).

Bortezomib-belinostat is being investigated in a phase I trial of patients with advanced solid tumors or lymphomas (NCT00348985). The maximum tolerated dose (MTD) for this combination has not yet been established, although modest antitumor activity has been reported (26). A phase II study evaluating bortezomib-belinostat in relapsed and/or refractory MM (NCT00431340) was stopped early because of dose-limiting toxicity (DLT).

Two other HDACi, panobinostat and romidepsin, are also being assessed in combination with bortezomib in phase I trials enrolling patients with relapsed and/or refractory MM (NCT00431990). Both trials included dexamethasone at some point in the treatment, and accrued patients previously treated with bortezomib. Several responses including one immunofixation negative complete response (CR), one VGPR, and three PR were noted in 14 patients entered in early cohorts of the panobinostat trial (27). Bortezomib-romidepsin and dexamethasone treatment induced a CR in one patient and at least a PR in 7 out of 10 patients (70%), although transient thrombocytopenia was problematic (28).

Kinase inhibitors

Cyclin-dependent kinase inhibitors alvocidib and PD0332991. Alvocidib is a small molecule, pan-cyclin-dependent kinase (CDK) inhibitor that can induce cell cycle arrest and apoptosis, as well as downregulate cyclin D1 and vascular endothelial growth factor (VEGF). Alvocidib was the first potent CDK inhibitor to enter clinical trials and has shown promising activity in patients with CLL (29).

The combination of bortezomib and alvocidib has been evaluated in hematopoietic cell lines using clinically achievable levels of both agents. In Bcr/Abl-positive chronic myelogenous leukemia cell lines, sensitive and resistant to imatinib, bortezomib-alvocidib synergistically increased mitochondrial dysfunction and apoptosis through inactivation of the NF-κB and signal transducer and activator of transcription 3 and 5 (STAT3 and STAT5) pathways (30). Only the combination, not the single agents, induced apoptosis, decreased myeloid cell leukemia 1 protein (Mcl-1) expression, and increased proapoptotic Bak...
in anaplastic large cell lymphoma cell lines, and similar results were noted in other leukemic (31) and MCL lines (32). Sequence-dependent addition of bortezomib prior to alvocidib enhanced apoptosis induction in MM cell lines compared with single agent treatment (33).

The bortezomib-alvocidib combination regimen is being assessed in a phase I, dose-escalation study treating patients with recurrent or refractory indolent B-cell neoplasms (NCT00082784). Twice weekly bolus and hybrid infusion schedules of alvocidib, following bortezomib administration, are being tested. An MTD has been established for the bolus administration regimen, and encouraging antitumor activity included responses in bortezomib-refractory patients: two lymphoma patients had a CR and seven (five MM and two NHL) had a PR (34). Similar assessment of the hybrid alvocidib administration schedule is ongoing.

PD0332991 is an orally bioavailable selective inhibitor of CDK 4 and CDK 6. Proteasome inhibitors and PD032991 have synergistic antitumor activity in MM models (35). A phase I study in relapsed and/or refractory MM patients evaluating two different administration schedules is underway (36).

**Sorafenib and sunitinib.** Sorafenib is an oral multikinase inhibitor of RAF kinase, VEGF receptors 1, 2, and 3, platelet-derived growth factor receptor β (PDGFRβ), Flt-3, c-Kit, and RET receptor tyrosine kinases, which is approved for the treatment of advanced renal cell carcinoma (37) and hepatocellular carcinoma (38). Sorafenib can inhibit tumor growth and angiogenesis, as well as induce apoptosis in a cell context-specific manner, through either Raf-MAP/ERK kinase (MEK)-mitogen-activated protein kinase (MAPK)–dependent or –independent pathways. The bortezomib-sorafenib combination synergistically induced a marked increase in mitochondrial injury and apoptosis, requiring Akt inhibition, in multiple human tumor cell lines (39).

The combination of bortezomib-sorafenib is being investigated in two phase I trials. A study in advanced solid tumor patients (NCT00303797) assessed sorafenib and twice weekly bortezomib. Full doses of either agent could not be administered; an MTD of sorafenib 200 mg twice daily, and bortezomib 1 mg/m² was identified. DLT included abdominal pain with hyperamylasemia and vomiting. One patient with renal cancer had a PR, and four patients were reported to have stable disease (40). Administration of bortezomib on a weekly schedule in combination with sorafenib in patients with relapsed or refractory MM is being assessed in a second trial (NCT00536575).

Sunitinib is another oral multikinase inhibitor with a wide spectrum of inhibitory activity [including VEGFR, PDGFR, fibroblast growth factor receptor (FGFR), c-kit, FLT3, and others], which has been approved for the treatment of renal cell carcinoma and gastrointestinal stromal tumor (41). Bortezomib and sunitinib are being evaluated in a phase I solid tumor patient study (NCT00720148), and are being administered in a 6-week cycle with 4 weeks of drug administration followed by 2 weeks of rest.

**Other kinase inhibitors.** Several other kinase inhibitors that induce enhanced antitumor activity with bortezomib in preclinical models are also being investigated in early-phase clinical trials. Bortezomib is being evaluated with temsirolimus (CCI-779), which is approved for the treatment of renal cell cancer (42), and everolimus (RAD001): two small molecule inhibitors of the mammalian target of rapamycin (mTOR) kinase, which is an intermediate of the phosphoinositide 3-kinase (PI3K)/Akt/mTor signaling pathway. Both temsirolimus and everolimus have shown significant in vitro antitumor and in vivo cytostatic activity in xenograft models of NHL and MM, alone, and in combination with bortezomib (43, 44). Bortezomib-temsirolimus, both administered on a weekly schedule, is being evaluated in a phase I–II trial in relapsed and/or refractory MM (NCT00483262). The identified MTD was temsirolimus 25 mg and bortezomib 1.6 mg/m², and the overall response (CR + PR + minimal response) was 33% (5 out of 15 patients) in patients who had all been previously treated with bortezomib (45). Bortezomib is also being evaluated in combination with the orally bioavailable mTor inhibitor everolimus, in a phase I study of patients with relapsed and/or refractory MCL and other indolent lymphomas (NCT0067112).

Proteasome inhibition may activate epidermal growth factor receptor (EGFR)–dependent mitogenic signaling and sensitize cells to EGFR inhibition. The effects of co-treating NSCLC cell lines with bortezomib and either gefitinib, erlotinib, or cetuximab have been examined in EGFR-expressing cell line models, and, in some cases, the combinations have shown synergistic activity (46, 47). A phase I clinical trial combining bortezomib with cetuximab in advanced solid tumors (NCT00622674) and a phase II study of bortezomib-erlotinib in relapsed and/or refractory NSCLC (NCT00283634) have been initiated. The latter trial was closed on the basis of insufficient antitumor activity (48).

A phase I study combining dasatinib with bortezomib is being conducted in patients with relapsed and/or refractory MM (NCT00560352). Dasatinib, a multitargeted inhibitor of c-abl, src family proteins, EphA2, and btk, is approved for the treatment of chronic myelogenous leukemia (49). Preclinical studies of this combination in myeloma cell lines indicate there is synergistic antitumor activity (50), and a phase I trial in MM (NCT00560352) is ongoing.

Perifosine (KRX-0401) is an orally bioavailable signal transduction modulator with multiple effects, including inhibition of Akt. The combination of perifosine and bortezomib has been assessed in myeloma and prostate cell line models (51–53). Perifosine is being investigated in combination with bortezomib ± dexamethasone in a phase I–II study in patients with MM, all of whom are relapsed or refractory to previous bortezomib therapy (NCT00401011). Results from this study indicate that perifosine-bortezomib ±dexamethasone) is highly active and well tolerated; overall response for 57 evaluable patients was 40%, including 4% who achieved a CR (54).
<table>
<thead>
<tr>
<th>Class and/or agent</th>
<th>Study and tumor types</th>
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<tbody>
<tr>
<td><strong>HDACi</strong></td>
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<tr>
<td>Vorinostat</td>
<td>• Phase I dose-escalation study in metastatic or unresectable solid tumors</td>
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<td></td>
<td>• Phase I dose-escalation study in relapsed or refractory MM</td>
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<td>• Phase I dose-escalation study in surgically resectable stage IB-IIIA NSCLC</td>
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<td>• Phase I dose-escalation study in advanced MM</td>
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<td>• Phase II study in progressive, recurrent glioblastoma</td>
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<td></td>
<td>• Phase II study in MCL and diffuse large B-cell lymphomas</td>
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<td></td>
<td>• Phase II study in NSCLC</td>
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<tr>
<td>Belinostat</td>
<td>• Phase II study in relapsed and/or refractory MM</td>
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<tr>
<td>Panobinostat</td>
<td>• Phase II study in relapsed and/or refractory MM</td>
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<td>Romidepsin</td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<td></td>
<td>• Phase II study in relapsed and/or refractory MM with prior bortezomib therapy</td>
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<tr>
<td><strong>Kinase inhibitor</strong></td>
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<tr>
<td>Alvocidib</td>
<td>• Phase I dose-escalation study in recurrent or refractory indolent B-cell neoplasms</td>
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<tr>
<td>PD0332991 (CDK4/6 inhibitor)</td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<td>Sorafenib</td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<td>Sunitinib</td>
<td>• Phase I dose-escalation study in chemorefractory advanced solid tumors</td>
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<td>Temsirolimus</td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<td>Everolimus</td>
<td>• Phase I dose-escalation study in relapsed and/or refractory MCL, other indolent non-Hodgkin’s lymphoma</td>
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<td>Erlotinib</td>
<td>• Phase II study in relapsed and/or refractory metastatic NSCLC</td>
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<tr>
<td>Cetuximab</td>
<td>• Phase I dose-escalation study in advanced solid tumors</td>
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<td>Dasatinib</td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<tr>
<td>Perifosine</td>
<td>• Phase I–II study in relapsed and/or refractory MM (patients with prior progression on bortezomib treatment)</td>
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<td><strong>Farnesyl transferase inhibitor</strong></td>
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<tr>
<td>Tipifarnib</td>
<td>• Phase I dose-escalation study in advanced acute leukemias or chronic myelogenous leukemia in blast phase</td>
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<td>• Phase I–II study in newly diagnosed acute myeloid lymphoma ineligible for cytotoxic chemotherapy or in first relapse</td>
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<td>• Phase I dose-escalation study in relapsed and/or refractory MM</td>
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<td><strong>HSP90 chaperone inhibitor</strong></td>
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<td>Tanespimycin</td>
<td>• Phase I dose-escalation study in advanced solid tumors or lymphoma</td>
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<td>• Phase I dose-escalation study in relapsed and/or refractory hematologic malignancies</td>
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<td>• Phase III study in MM after first relapse</td>
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<td></td>
<td>• Phase II–III study in relapsed and/or refractory MM (at least three prior therapies)</td>
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<tr>
<td>AUY-922</td>
<td>• Phase I–II study in relapsed and/or refractory MM (one prior therapy)</td>
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<td><strong>Other targets</strong></td>
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<tr>
<td>Pan-Bcl-2 family inhibitor</td>
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<tr>
<td>obatoclax</td>
<td>• Phase I–II study in MCL</td>
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<td></td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<td></td>
<td>• Phase I dose-escalation study in aggressive relapsed and/or recurrent non-Hodgkin’s lymphoma</td>
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<td>DNA methyltransferase inhibitor</td>
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<td>azacytidine</td>
<td>• Phase I dose-escalation study in relapsed and/or refractory acute myeloid leukemia and myelodysplastic syndromes</td>
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<td><strong>Monoclonal antibodies</strong></td>
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*(Continued on the following page)*
Other kinase inhibitors that have been investigated with bortezomib in preclinical studies include: BIRB796/doramapimod, a p38 MAPK inhibitor; BIBF 1000, a receptor kinase inhibitor of PDGF, basic FGF, and VEGF; enzastaurin, a protein kinase \(\beta \) inhibitor; SCIO-469, a p38 \(\alpha \) MAPK inhibitor; and LSN2322600, another p38 MAPK inhibitor (Supplementary Table S3).

**Farnesyltransferase inhibitors**

Farnesyltransferase inhibitors (FTI) inhibit the activation of Ras, and many other target proteins that are substrates of the farnesyltransferase enzyme, and regulate signal transduction, tumor cell proliferation, and cell growth. Tipifarnib (R115777, Zarnestra) is an orally bioavailable FTI with documented clinical activity in acute myeloid leukemia and myelodysplastic syndrome (55). In preclinical studies with bortezomib, tipifarnib, and another FTI, lonafarnib demonstrated additive and/or synergistic activity in head and neck squamous cell carcinoma cells (56), as well as several hematologic malignancies including MM, AML, and MCL models (57–59). Increased apoptosis in MM cell lines and primary patient cells compared with either agent alone (58) was noted, as was a sequence dependency with bortezomib followed by lonafarnib, which was critical for optimal induction of apoptosis (60).

Several ongoing early-phase clinical trials are investigating bortezomib-tipifarnib combination therapy in hematologic malignancies. A phase I trial of bortezomib-tipifarnib in relapsed acute leukemia blast phase chronic myelogenous leukemia (NCT00383474) established an MTD at full doses of both agents (tipifarnib 600 mg twice daily and bortezomib 1.3 mg/m²). Two patients achieved a CR and four had stable disease (61). Another phase I–II study in AML (NCT00510939) is evaluating this combination and correlating clinical outcomes with results of a pretreatment two gene expression signature for tipifarnib sensitivity (62, 63). A phase I trial is being conducted in patients with relapsed and/or refractory MM, and two patients have evidence of response at doses below the MTD (64).

**Heat-shock protein 90 chaperone inhibitors**

Heat-shock protein 90 (HSP90), an abundant cellular protein, is a chaperone that stabilizes various client proteins (e.g., Flt3, Akt, c-Raf, Bcr-Abl, and c-Src), which are key intermediates in cancer survival and proliferative pathways (65). Several HSP90 inhibitors have been developed, including the natural product benzoquinone ansamycin derivatives (e.g., tanespimycin, alvespimycin, retaspimycin, CNF1010), and other structurally unrelated synthetic compounds (AUY922, an isoxazole derivative, SNX-5422, STA-9090, AT13387, PU-H71; ref. 66). Preclinical studies

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**Table 1. Ongoing clinical trials program of bortezomib in combination with novel targeted agents (Cont’d)**

<table>
<thead>
<tr>
<th>Class and/or agent</th>
<th>Study and tumor types</th>
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<tbody>
<tr>
<td>Elotuzumab (anti-CS1)</td>
<td>• Phase I–II study in relapsed and/or refractory MM</td>
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<tr>
<td>Mapatumumab (anti-TRAIL-R1)</td>
<td>• Phase II study in relapsed and/or refractory MM</td>
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<tr>
<td>CNTO-328 (anti-IL6)</td>
<td>• Phase II study in relapsed and/or refractory MM</td>
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<tr>
<td>Bevacizumab (anti-VEGF)</td>
<td>• Phase I dose-escalation study in solid tumors, lymphoma, myeloma</td>
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<tr>
<td>Rituximab (anti-CD20)</td>
<td>• Phase II study in relapsed and/or refractory MM</td>
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<tr>
<td>Trastuzumab (anti-HER2)</td>
<td>• Phase I dose-escalation study in HER2-expressing breast cancer</td>
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**Table 2. Histone deacetylase inhibitors combined with bortezomib in ongoing clinical trials**

<table>
<thead>
<tr>
<th>HDACi</th>
<th>Class I and II inhibitor or group</th>
<th>Subclass or type of inhibitor</th>
<th>Route of administration</th>
<th>Stage of development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>Hydroxamic acid</td>
<td>Pan-HDACi</td>
<td>Oral</td>
<td>Approved for cutaneous T-cell lymphoma therapy</td>
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<tr>
<td>Belinostat</td>
<td>Hydroxamic acid</td>
<td>Pan-HDACi</td>
<td>Intravenous (oral)</td>
<td>Phase II</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>Hydroxamic acid</td>
<td>Pan-HDACi</td>
<td>Oral (intravenous)</td>
<td>Phase II</td>
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<tr>
<td>Romidepsin</td>
<td>Cyclic depsipeptide</td>
<td>Class I-selective HDACi</td>
<td>Intravenous</td>
<td>Phase II</td>
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of bortezomib combined with geldanamycin, tanespimycin, and retaspimycin have been reported in solid tumor and hematologic tumor models, and the combination seems to have synergistic antitumor activity (67–71), except bortezomib and ALIY922, reportedly because of the potent low nanomolar efficacy of this HSP90 inhibitor in the MM model tested (72). Several mechanistic insights have been gained from these in vitro studies. Tanespimycin and bortezomib treatment of MM cells results in enhanced intracellular accumulation of ubiquitinated proteins because of synergistic inhibition of the proteasome chymotryptic active site. HSP90 inhibitors and bortezomib also seem to have complementary and distinct roles in undermining the unfolded protein response in cancer cells, resulting in a proapoptotic signal that culminates in cell death.

Bortezomib-tanespimycin combination therapy has been evaluated in phase I trials in solid tumor and MM patients. Encouraging activity with manageable toxicities was shown in heavily pretreated patients with relapsed and/or refractory MM, even in patients refractory to bortezomib (73). These clinical results have led to initiation of a phase III trial (TIME-1, NCT00546780) in MM patients after first relapse comparing bortezomib-tanespimycin to bortezomib alone. A supportive phase II–III study (TIME-2) is a three-arm study of bortezomib and different tanespimycin doses, which is accruing patients who have relapsed after at least three prior regimens that must have included bortezomib and lenalidomide.

Pan-Bcl-2 family inhibitors

Obatoclax. The Bcl family of proteins, which mediates tumor cell survival and chemoresistance, is dysregulated in many malignancies and has emerged as an important therapeutic target. Obatoclax is a novel, small molecule mimetic of proapoptotic BH3-only protein, which can interact with the BH3-binding groove of Bcl-2 family proteins (including Mcl-1), inhibit the interaction between pro- and anti-apoptotic proteins, and induce apoptosis (74). In addition, obatoclax induces an S-G2 cell cycle block and initiates autophagy (75, 76). In preclinical studies, obatoclax and bortezomib had synergistic antitumor activity against MCL and CLL cell lines, purportedly through a combination of decreasing bortezomib-induced Mcl-1 and enhancing Noxa-mediated displacement of Bak from Mcl-1 (77, 78). Bortezomib is being investigated with obatoclax in three phase I or phase I–II studies in patients with relapsed and/or refractory MCL (NCT00407303), MM (NCT00719901), and NHL (NCT00538187). Preliminary data from the MCL trial indicate that the combination is active (29% of patients achieved ≥PR) and has acceptable tolerability in heavily pretreated patients (79).

Other novel targeted therapies

Early-phase clinical trials combining bortezomib with monoclonal antibodies directed at a variety of targets are also ongoing. Elotuzumab is a humanized monoclonal antibody that targets CS1, a cell surface glycoprotein expressed on myeloma cells (80), and synergistically inhibits tumor growth with bortezomib in tumor models (81). Mapatumumab, a monoclonal antibody directed at the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-R1 receptor (82), enhances apoptosis in TRAIL-R1-expressing cell lines when combined with bortezomib (83). Interleukin-6 (IL-6) is an important signaling intermediate in MM that is critical for the HSP response; combinations of CNTO328, a chimeric antibody directed at IL-6, and bortezomib have synergistic cytotoxic activity in IL-6-dependent and IL-6–independent myeloma cell lines (84). Clinical trials evaluating the combination of bortezomib with these three antibodies in MM are ongoing (85).

VEGF clearly plays a critical role in the pathogenesis of solid tumors and myeloma (86), and preclinical studies of G6.31, a VEGF-directed murine-human monoclonal antibody, in combination with bortezomib, produced a marked increase in antimumeloma activity (87). The bevacizumab-bortezomib regimen is being evaluated in early-phase glioma, renal cell cancer, NSCLC, and MM studies. Phase II studies of bortezomib-rituximab are being conducted in Waldenstrom’s macroglobulinemia or indolent NHL patients, on the basis primarily of clinical evidence of single agent activity; additive antitumor activity was seen with this combination only in a CLL model (88). A phase II study of bortezomib-trastuzumab in human epidermal growth factor receptor 2 (HER2)-positive breast cancer patients was initiated, on the basis of preclinical evidence indicating synergistic activity of the two agents in breast cancer cell lines and bortezomib overcoming trastuzumab resistance (89, 90).

Several studies are evaluating bortezomib in combination with immunomodulatory drugs (IMiD), such as thalidomide or lenalidomide (91, 92). The rationale for these combinations is provided by preclinical studies showing that IMiDs potentiate the proapoptotic effect of bortezomib on MM cells through multiple mechanisms of action involving caspase-8 activation; MM cell sensitization to Fas-mediated apoptosis; downregulation of the caspase-8 inhibitors, cellular inhibitor of apoptosis protein-2 (cIAP-2) and FLICE inhibitory protein (FLIP); and downregulation of NF-κB activity, among other anti-MM effects (93). The efficacy of combining bortezomib and lenalidomide has been shown in a phase I study in patients with relapsed and/or refractory MM, which reported the combination to be well tolerated and to have promising activity with durable responses (94). Bortezomib-lenalidomide combinations are subsequently under study in the phase II and III settings.

Other novel targeted agents are in development and have been investigated in combination with bortezomib in preclinical studies. Supplementary Table S3 provides an overview of these agents and their mechanisms of action.

Discussion

The progress in clinical and translational research on bortezomib is providing increasing insights into the cellular
and molecular mechanisms underlying the antitumor efficacy of this proteasome therapeutic agent. Important recent observations have included gene expression signatures associated with sensitivity to bortezomib (95), impact of bortezomib on cancer stem cell biology (96), and bortezomib’s potent role as an inducer of the endoplasmic reticulum stress responses (22). In parallel, improved understanding of the molecular pathways important in cancer cell biology has led to the development of an extraordinary number of novel, targeted investigative agents, including the second-generation proteasome inhibitors carfilzomib (97), marizomib (98), MLN9708 (99), and CEP18770 (100), which may expand the limited universe of proteasome-directed therapies and afford different clinical characteristics from bortezomib. This convergence has led to the strategy of cotargeting signaling pathways or intermediates in the cancer cell by developing treatment regimens combining bortezomib and a targeted agent.

This review encompasses a diverse group of approximately two dozen therapeutic agents currently being assessed with bortezomib in more than 40 ongoing early-phase clinical trials. Preclinical in vitro or in vivo data, using clinically achievable concentrations of drug, have shown evidence of synergistic antitumor efficacy, providing a supportive rationale for most of these doublet combinations. The majority of the studies are evaluating patients with hematologic malignancies, primarily MM and lymphoma, on the basis of the proven activity of bortezomib in MM and MCL. Phase III studies of two combinations, bortezomib-vorinostat and bortezomib-tanespimycin, have been initiated, and additional randomized studies are planned. Most of the doublet combinations seem to be well tolerated, and single agent concentrations of both drugs are generally recommended for most, but not all, phase II studies. Encouraging clinical responses have been noted in patients with solid tumors as well as MM patients, and preliminary data also indicate a potential reduction in toxicities with certain combinations, such as the potential neuroprotective effect of tanespimycin with bortezomib (73).

Although trials of most combinations are still in early stages, and conclusions about the overall effectiveness of each doublet remain open, it is clear with the emergence of additional novel targeted agents and supportive preclinical data that further assessment of this therapeutic strategy will be considered. Translational research conducted as part of the ongoing trials may inform prioritization of specific combinations. This approach may facilitate the development of combination regimens optimized for specific tumor subtypes, thus providing the potential for tailored therapy in individual patients on the basis of certain molecular and genetic characteristics of their disease.

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