Molecular Pathways: Targeting Proteasomal Protein Degradation in Cancer
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Abstract
With the approval by the U.S. Food and Drug Administration of bortezomib for the treatment of multiple myeloma and mantle cell lymphoma, the proteasome was clinically validated as a target in oncology. The proteasome is part of a complex cellular pathway that controls the specificity and rate of degradation of the majority of proteins in the cell. The search for additional drug targets in the proteasomal pathway is ongoing. In parallel, the next generation of proteasome inhibitors, exhibiting some properties distinct from that of bortezomib, are currently being studied in clinical trials. The key question will be whether these distinctions can improve upon the clinical efficacy and safety standards established by bortezomib and refine our understanding of the mechanism by which proteasome inhibitors are effective in the treatment of cancer.

Clinical validation of the proteasome as a therapeutic target
Cancer cells are more sensitive to proteasome inhibition and apoptotic induction than nontransformed cells for reasons that are not entirely understood (7, 8), and proteasome inhibitors have shown single-agent activity in several different animal tumor models. Clinical validation of the proteasome as a cancer therapeutic target was established by bortezomib (Velcade; Millennium Pharmaceuticals/Takeda Pharmaceuticals; 9). Bortezomib, the first proteasome inhibitor to enter clinical trials, was granted accelerated approval by the U.S. Food and Drug Administration (FDA) in 2003 after showing impressive single-agent responses in patients with relapsed and refractory myeloma (10–13). Bortezomib has since been approved for the treatment of mantle cell lymphoma (MCL; refs. 14–17), as well as for the treatment of newly diagnosed multiple myeloma (18, 19). Although bortezomib is an effective treatment, proteasome inhibitors with reduced toxicity, improved efficacy, and oral bioavailability are still needed (20, 21). Inhibitors that have efficacy in other tumor types are also needed (22). These objectives have spawned the development of several “next-generation” proteasome inhibitors, of which 5 have entered clinical development since the approval of bortezomib.

Next-generation proteasome inhibitors in clinical development
The proteasome inhibitors currently in the clinic are derived from 3 structural classes: dipetide boronic acids [represented by bortezomib (23), CEP-18770 (24), and MLN9708 (25)]; β-lactones (represented by NPI-0052, a marine microbial natural product related to omuralide; ref. 26); and peptide epoxyketones [represented by carfilzomib (PR-171; ref. 27), a tetrapeptide epoxyketone related to the natural product epoxomicin (28) and ONX-0912, a tripeptide analogue (29)].

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Each inhibitor class reacts with the proteasome N-terminal threonine active sites by a distinct mechanism (30). Peptide boronic acids form a slowly reversible tetrahedral adduct with the γ-OH group of the catalytic threonine (23, 31). For the β-lactone NPI-0052, attack of the lactone ring by the catalytic γ-OH results in formation of an ester bond and an intramolecular rearrangement that makes the inhibition irreversible (32–34). For peptide epoxyketones, the peptide portion binds to the substrate-binding pocket of the proteasome, allowing the epoxyketone to interact stereospecifically with both the γ-OH and the α-amino groups of the catalytic threonine to form 2 covalent bonds, making the inhibition irreversible and selective (35).

Interestingly, proteasome activity recovers at the same rate with irreversible inhibitors as with slowly reversible inhibitors (27, 36), presumably because of induction of de novo proteasome synthesis (37).

The 3 threonine proteases are defined by their substrate selectivity: chymotrypsin-like–β5 subunit (CT-L); trypsin-like–β2 subunit (T-L); and postacidic or caspase-like–β1 subunit (C-L). The proteasome inhibitors in clinical development have the greatest potency for the CT-L active site of the proteasome (24, 27, 36, 38, 39), but they differ in their activity against the other catalytic sites. The development of compounds with different selectivity profiles will help address the question of what combination of proteasome site inhibition provides maximal antitumor effects and the best therapeutic index.

Clinical–Translational Advances

Preclinical antitumor activity

NPI-0052. NPI-0052 is active against a variety of tumor cell types (40, 41), including primary plasma cells from bortezomib-resistant patients with multiple myeloma. When NPI-0052 and bortezomib were compared head to head in xenograft studies using the twice-weekly (days 1 and 4) bortezomib clinical-dosing schedule, the activity of the 2 compounds was comparable in a multiple myeloma model (38, 42), whereas bortezomib displayed better efficacy than NPI-0052 in a prostate cancer model (36). In a disseminated lymphoma model, once-weekly bortezomib had superior efficacy to twice-weekly NPI-0052 (36, 40).

NPI-0052 is orally active, as are CEP-18770, MLN9708, and ONX-0912. However, unlike any of the other proteasome inhibitors (27, 36, 39, 43), NPI-0052 penetrates the blood–brain barrier and inhibits brain proteasome CT-L activity by >90% (36). Abnormalities in the ubiquitin–proteasome system have been linked to neurodegenerative diseases (44, 45), raising potential safety concerns around long-term treatment with NPI-0052.

CEP-18770. CEP-18770 has in vitro antitumor activity similar to that of bortezomib in hematologic and solid tumor cell lines, as well as in primary plasma cells from patients with multiple myeloma. In addition, CEP-18770 had improved activity relative to bortezomib in a subcutaneous multiple myeloma xenograft model (RPMI-8226 cells) using a twice-weekly dosing schedule on days 1 and 4, and it had comparable activity in a disseminated multiple myeloma model using ARP-1 cells (39).

Carfilzomib. Carfilzomib (PR-171) has in vitro activity across a range of tumor cell types, including multiple myeloma cells that are resistant to bortezomib (27, 46, 47). Carfilzomib is more active than bortezomib when cells are treated for short periods that mimic in vivo single-dose exposure; however, both compounds have greater activity when cells are exposed for prolonged periods of time (27, 46). To achieve prolonged inhibition in vivo, carfilzomib
was dosed daily and was well tolerated at doses that inhibited more than 80% of proteasome CI-L activity in blood and tissues. This finding is in contrast to bortezomib, in which a two-weekly clinical-dosing schedule on days 1 and 4, which allows full recovery of proteasome activity between doses, was selected, in part, because of excessive toxicity associated with more frequent dosing schemes (9). In xenograft studies, dosing of carfilzomib for 2 consecutive days (days 1 and 2) on a weekly schedule was superior to either twice-weekly dosing on days 1 and 4 or once-weekly dosing (27), supporting the in vitro observation that delaying complete proteasome recovery results in superior antitumor responses.

**MLN9708.** The boronate MLN9708 has a similar profile to that of bortezomib in active site selectivity and potency in cytotoxicity assays, but it has a shorter proteasome dissociation half-life. In vivo, MLN9708 disseminates more widely to tissues than does bortezomib, resulting in greater blood and plasma exposures at the maximum tolerated dose and greater pharmacodynamic activity and efficacy in tumors. In contrast to bortezomib, MLN9708 has oral bioavailability, and it is efficacious and tolerated when dosed daily (25).

**ONX-0912.** ONX-0912 (PR-047), an analogue of carfilzomib, was developed as an orally bioavailable peptide epoxyketone proteasome inhibitor (29). ONX-0912 has similar potency to carfilzomib in cytotoxicity assays and has equivalent antitumor activity to i.v.-administered carfilzomib in multiple human tumor xenograft and mouse syngeneic models. It is well tolerated with repeated daily oral administration at doses that result in >80% proteasome inhibition in most tissues (48).

**Mechanism of action**

Proteasome inhibitors block the global turnover of proteins, but there may be 1 or more key proteins with turnover that, when blocked, causes caspase activation and apoptosis in cancer cells. It has been suggested that blocking the prosurvival NF-kB pathway, by blocking the turnover of the inhibitory protein I-kB, is key for inducing apoptosis. It has also been shown that the proapoptotic factor NOXA, which interacts with the antiapoptotic factor Mcl-1 as well as other antiapoptotic factors in the Bcl-2 family and causes the release of cytochrome c into the cytosol and activation of apoptosis, is induced in tumor cells upon treatment with proteasome inhibitors. In multiple myeloma, the tumor cells secrete great quantities of monoclonal antibody (M protein) and are dependent on the induction of the unfolded protein response (UPR) pathway to reduce the accumulation of misfolded proteins in the endoplasmic reticulum; otherwise the cells undergo apoptosis. Proteasome inhibition interferes with the UPR pathways and blocks the destruction of misfolded proteins by the proteasome (49, 50).

**Clinical experience with proteasome inhibitors**

**Bortezomib approvals in multiple myeloma and mantle cell lymphoma.** Bortezomib has single-agent efficacy in multiple myeloma, resulting in 30% to 40% partial response rates in patients who have relapsed from prior therapies (a partial response is ≥50% decrease in M protein, the monoclonal antibody secreted by transformed plasma cells and present in blood and urine of patients with myeloma). This finding enabled rapid approval of bortezomib by the FDA in 2003 (11, 12). The combination of bortezomib and pegylated liposomal doxorubicin was shown to be superior to single-agent bortezomib (51), leading to the FDA approval of this combination in 2007. In newly diagnosed multiple myeloma, the addition of bortezomib to the standard therapy of melphalan and prednisone (MP) improved response rate (71% vs. 35%) and progression-free survival (24 months vs. 16.6 months), and it conferred an overall survival advantage relative to MP alone. On the basis of this study, bortezomib received FDA approval in 2008 in newly diagnosed patients (20, 21). In a phase I and II study in front-line patients with myeloma, bortezomib addition to lenalidomide and dexamethasone gave a 100% response rate, with 74% of responses being very good partial response or better (a very good partial response is ≥90% decrease in M protein), showing that the combination of these drugs is highly effective (52).

Bortezomib also has single-agent activity in relapsed MCL. A single-arm phase II study, in which the response rate was 31% and the duration of response was 9.3 months, was the basis for approval of bortezomib to treat MCL (15, 16).

**Clinical pharmacodynamics and safety of bortezomib.** In patients treated with bortezomib, inhibition of CI-L activity in blood reaches a plateau at 65% to 70% (10), considerably less than the >90% proteasome inhibition seen in animal studies (43). The bortezomib clinical-dosing schedule (days 1, 4, 8, and 11 of a 21-day cycle) allows for complete recovery of proteasome activity between doses. The most notable toxicities observed with bortezomib include hematologic toxicities (thrombocytopenia and neutropenia), peripheral neuropathy, and gastrointestinal toxicities. Bortezomib-induced thrombocytopenia is transient in nature with recovery seen between dosing cycles (20); a block of platelet budding from megakaryocytes has been proposed as the underlying mechanism. Dose-dependent peripheral neuropathy (frequently painful) was reported in the initial phase II bortezomib trials, with an incidence of 37% at the recommended 1.3 mg/m² dose (14% grade 3; refs. 9, 21, 53). This toxicity is now managed by dose reduction or withholding doses, but the impact of these modifications on the antmyeloma activity of bortezomib is not fully known.

**Carfilzomib.** The most advanced of the next-generation proteasome inhibitors is carfilzomib, which entered the clinic in 2005. Single-agent responses were seen in 2 phase I studies with consecutive-day dosing schedules (54, 55) in which the drug was well tolerated and induced >80% proteasome inhibition in blood. On the basis of these data, a large phase II single-arm trial was conducted in patients with myeloma who had failed all available therapies, including bortezomib. Despite the fact that patients had a median of 5 prior lines of multdrug therapy, 24% of
patients had a partial response and 37% of patients had a minimal response (≥25% decrease in M protein) or better to carfilzomib. In responding patients, the median overall survival was 20.7 months (56). Transient thrombocytopenia was observed (similar to that noted with bortezomib), but peripheral neuropathy rates were greatly reduced relative to bortezomib, suggesting that neuropathy may be an off-target side effect (57). An even higher response rate of 53% partial response was seen in patients with relapsed myeloma who had not received prior bortezomib. In newly diagnosed patients treated with a combination of carfilzomib, lenalidomide, and dexamethasone, the complete response rate (absence of detectable M protein and ≤5% myeloma plasma cells in bone marrow) was 61%, and 83% of patients had a very good partial response or better (58). Carfilzomib is currently in a randomized phase III registration trial in patients with relapsed myeloma, comparing carfilzomib with lenalidomide and dexamethasone to lenalidomide and dexamethasone alone.

NPI-0052 and CEP-18770. NPI-0052 entered the clinic with a phase I trial in 2006 in patients with solid tumors and in patients with lymphoma. Stable disease was observed in some patients, and proteasome inhibition in blood exceeded that observed with bortezomib (59). Additional phase I trials with NPI-0052 in myeloma and other malignancies are currently ongoing.

CEP-18770 entered the clinic in 2007 in a phase I study in patients with solid tumors and in patients with lymphoma to whom the drug was administered on the same schedule as bortezomib (days 1, 4, 8, and 11 of a 21-day cycle). Phase I and II trials in relapsed refractory myeloma, with CEP-18770 given alone or in combination with lenalidomide and dexamethasone, are currently ongoing.

MLN9708 and ONX-0912. In a series of phase I trials initiated in 2009, MLN9708 had substantial oral bioavailability and was well tolerated when dosed on a once-weekly or twice-weekly schedule. In relapsed and refractory patients who had received prior bortezomib, interim data from a dose-escalation study reported at the International Myeloma Workshop in May 2011 showed that 2 out of 35 patients had partial responses. A phase I trial with ONX-0912 in patients with solid tumors started in 2010, and interim results reported at the American Society of Clinical Oncology in 2011 showed that, at doses that were well tolerated, >80% proteasome inhibition in blood could be achieved on a once-daily dosing schedule for 5 consecutive days (60).

Conclusions

The approvals of bortezomib for the treatment of multiple myeloma and MCL have provided clinical validation of the proteasome as a therapeutic target. The next generation of proteasome inhibitors exhibits both similarities and differences relative to bortezomib in terms of mechanism, selectivity, and preclinical antitumor activity. The results of clinical trials with these new agents will increase our understanding of the role of the proteasome in cancer cells and, it is hoped, expand the role that proteasome inhibitors will play in the treatment of cancer.

Disclosure of Potential Conflicts of Interest

S.M. Molineaux: past employment, Onyx Pharmaceuticals (Proteolix); spouse was formerly a consultant for Onyx Pharmaceuticals.

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