Proteasome Inhibitor Drugs on the Rise

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Abstract

In May 2003, the U.S. Food and Drug Administration granted the proteasome inhibitor bortezomib (Velcade) fast-track status for the treatment of multiple myeloma. This landmark represented the first approval of a drug targeting the ubiquitin-proteasome system (UPS) for any indication. More recently, at the AACR Special Conference "Ubiquitin and Cancer: From Molecular Targets and Mechanisms to the Clinic" (Orlando, FL, January 18-22, 2006), it became evident that drug discovery in the UPS is experiencing another round of great excitement. The reason—new clinical applications found for bortezomib, along with the promised success of new types of proteasome inhibitors reaching the clinic. (Cancer Res 2006; 66(16): 7840-2)

The Proteasome, Bortezomib, and Multiple Myeloma

The 26S proteasome is a 2-MDa multisubunit protease that degrades most cytosolic, endoplasmic reticulum and nuclear proteins (1). The best understood mechanism for targeting proteins for proteasomal degradation involves conjugation to the 8-kDa protein, ubiquitin. The 26S proteasome is made up of a 20S core and a 19S regulatory complex. 19S chaperones unfold ubiquitin-tagged protein substrates and feed them through the cylinder-shaped 20S core, whose inner surface displays three pairs of proteolytic active sites. These sites are named after similarity of their cleavage specificity to chymotrypsin, trypsin, and caspases. Studies with mutants of budding yeast had originally suggested different roles for the three sites, with the chymotrypsin-like one being the most important for normal growth (2).

Small-molecule proteasome inhibitors are usually peptidomimetics whose COOH-terminal reactive groups take advantage of the unique catalytic mechanism of proteasome subunits (involving NH_2-terminal threonine nucleophiles) to achieve specificity (1). For example, the commonly used research reagent, MG-132 (Z-Leu-Leu-Leu-al), is a peptide aldehyde that reversibly inhibits the chymotryptic-like site of the proteasome but can also inhibit lysosomal cathepsins at higher concentrations. Other classes of inhibitors can achieve greater specificity and potency toward the proteasome, such as peptide boronates, lactacystin and epoxomicin, and thus have become the choice for development into drugs.

The first of these proteasome inhibitors to reach the clinic was the dipeptide boronate of Millennium Pharmaceuticals (Cambridge, MA), bortezomib, a slowly reversible inhibitor of the chymotryptic site. David Schenkein (Millennium Pharmaceuticals) started the session "Clinical and Preclinical Studies Targeting the Ubiquitin-Proteasome System," chaired by Alfred Goldberg (Department of Cell Biology, Harvard Medical School), by presenting the history of development of bortezomib. The drug candidate, which in preclinical studies had shown significant activity against a mouse xenograft model of human multiple myeloma, was initially evaluated for inhibition of the degradation of IκB-α. The hope was to prevent activation of nuclear factor-κB in tumor cells, with the rationale that this would lead to inhibition of the related intrinsic drug resistance, of expression of adhesion molecules for bone marrow residency, and of expression and secretion of the cytokines required for multiple myeloma growth in the bone marrow milieu. (More recent studies are suggesting that multiple factors probably contribute to therapeutic effects of bortezomib.) Durable responses and clinical benefit in phase II trials in relapsed refractory multiple myeloma led to Food and Drug Administration approval of bortezomib, which was then extended to relapsed multiple myeloma based on a phase III trial showing prolonged time to progression compared with conventional therapy.

Inhibition of the growth of myeloma cells isolated from patients required ~170 times lower drug concentrations than for normal peripheral blood mononuclear cells. The relative insensitivity of normal cells to bortezomib is also reflected in the clinic; maximum tolerated dose studies revealed that one can achieve ~80% inhibition of the chymotryptic site in patient blood leukocytes using a drug concentration at which patients experience little toxicity relative to standard chemotherapy. This low toxicity is still surprising to many researchers in the field, given the major role of the ubiquitin-proteasome system (UPS) in many cellular processes in all cell types, including roles in eliminating misfolded and aggregating proteins and in degrading unstable proteins that function as proapoptotic regulators, cell cycle regulators, or transcription factors. For example, at the AACR meeting, Goldberg discussed a possible explanation; his group showed that the selective inhibition of the chymotryptic site to a degree compared with that seen with bortezomib-treated patients led to <50% decrease in the breakdown of a model substrate *in vitro* and had little effect on overall protein degradation in cultured cells (see also ref. 3). Thus, limited inhibition of protein degradation may account for the unexpectedly lower toxicity of bortezomib toward nontumor cells.

New Clinical Studies and Candidate Second-Generation Drugs

The clear trend in the clinic is to now examine the efficacy of bortezomib in other types of cancer as reflected in the talks by Owen O'Connor (Memorial Sloan-Kettering, New York, NY), Kenneth Anderson (Dana-Farber Cancer Institute), and Schenkein. For example, phase II trials for refractory indolent and aggressive B-cell lymphoma, as well as mantle cell lymphoma, a non-Hodgkin's lymphoma with very poor overall survival, are showing positive results (O'Connor). Early studies in relapsed leukemia also showed promise.

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As reported during the conference, combination therapies play important roles in the new bortezomib trials as well. Importantly, the drug can be used in combination to sensitize or overcome resistance to DNA-damaging agents (Schenkein); together with the Hsp90 inhibitor 17-AAG, bortezomib led to responses even in patients with bortezomib-refractory multiple myeloma (Anderson); lenalidomide (a thalidomide analogue) is another new drug with shown activity in relapsed and refractory multiple myeloma, presumably acting through a different target. Consistent with the previous observation that lenalidomide and bortezomib synergize in promoting apoptosis in tissue culture, phase I trials with multiple myeloma show that these drugs can be combined at active doses, thus far exhibiting promising activity (Schenkein). Because the choice of drugs for combination therapies remains largely an empirical process, the striking results with bortezomib certainly argue for continued investments on studies aiming at building an underlying scientific basis for this clinical practice.

For reasons that remain unclear, initial studies with solid tumors, including colon cancer, have not been promising. There are, however, early signs of activity in a subset of patients with non–small cell lung cancer (Schenkein). Following the talks, there was discussion of recent data from others suggesting that bortezomib coupled with endoplasmic reticulum stress-mediated apoptosis or with aggresome inhibition may also have efficacy in pancreatic carcinoma (see also refs. 4, 5).

Some patients with advanced multiple myeloma experience peripheral neuropathy (6), which may have discouraged new drug discovery activities in this class, at least to some extent, due to such liabilities. It is exciting that phase I trials are now suggesting that this side effect may be relieved in the bortezomib-lenalinomide combined therapy (Schenkein and Anderson). The question that remains to be answered is whether neuropathy results from proteasome inhibition or whether it is due to an off-target effect. Betting on the latter possibility, some small companies have pursued the development of new types of antiproteasome drugs with alternative chemistries.

The preclinical development of two such novel proteasome inhibitors was presented at the meeting (Fig. 1). Anderson talked about salinosporamide A (NPI-0052; Nereus Pharmaceuticals, San Diego, CA) which, unlike bortezomib, is orally available. This natural product derivative resembles lactacystin and irreversibly targets all three active sites (7, 8). Despite such seeming promiscuity, NPI-0052 did not target other proteases tested, possibly due to chemical specificity toward the unique catalytic mechanism of the proteasome. Francesco Parlati presented PR-171 (Proteolix, South San Francisco, CA), an irreversible inhibitor of the chymotryptic site synthetically derived from epoxomycin, the most selective type of proteasome inhibitor known. Both NPI-0052 and PR-171 induced apoptosis in multiple myeloma cells resistant to bortezomib and to other therapies and showed effectiveness in animal models. As a result, investigational new drug applications for both drugs have been successfully filed and both are undergoing early phase clinical trials in multiple myeloma. Whether these compounds ultimately exhibit greater therapeutic indices than bortezomib, they represent critical new tools for understanding how factors like the degree of inhibition of the three proteolytic sites, reversibility, and overall protein breakdown translate into clinical response and toxicity.

Another main direction in the field that was discussed was the challenge of elucidating which of the many pathways downstream of proteasome inhibition are critical for response. Answering this question is also important because it may help explain the greater sensitivity of cancer cells to the compounds and because of the potential to identify novel therapeutic targets or markers for drug response and resistance in those pathways. Anderson presented data showing that apoptosis in response to bortezomib and to NPI-0052 involves distinct pathways, the latter relying on caspase-8 (see also ref. 8). Such differences can perhaps be explained by the distinct effects that these drugs have on the three proteolytic sites, together with Goldberg's observation that the degradation of model substrates can be affected differently by selective inhibition of each proteolytic site.

Perspectives

That the therapeutic approach of interfering with protein degradation is already validated, although the specificity of the UPS in cell biology and its role in disease were not fully appreciated

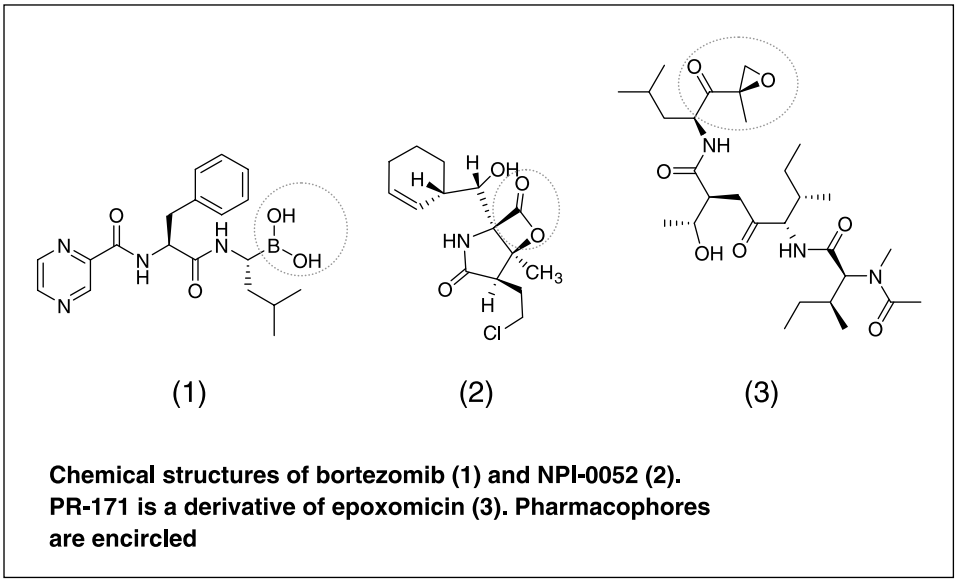

Figure 1. Proteasome inhibitor drugs. Chemical structures of bortezomib (*1*), salinosporamide A/NPI-0052 (*2*), and epoxomicin (*3*). PR-171 is a derivative of epoxomicin. *Encircled,* pharmacophores.

until recently, reflects the rapid evolution of the field. Clinical success with proteasome inhibitors is exciting not only for the hope it offers to patients but also because it strengthens the notion that modulating other steps on ubiquitin pathways might likewise be therapeutically successful. Drug discovery efforts are already being directed toward inhibiting substrate ubiquitylation by E3 ubiquitin ligases as well as the reverse reaction catalyzed by deubiquitylating enzymes (DUBs). As it became clear from other sessions at the meeting, the task is now to identify and validate new targets among the hundreds of E3s or DUBs, better understand their mechanism of action and substrate specificity, and screen or design small molecule inhibitors against these components.

Given the explosion of activities in the UPS field, in both basic and applied research, it is not unreasonable to expect that, like for protein kinases a decade or so ago, much more excitement is yet to come from targeting protein degradation for drug discovery and development.

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