Ubiquitin-Proteasome Pathway

The ubiquitin-proteasome pathway is the principal pathway for intracellular protein degradation\(^1,2\) (Fig 1). This pathway selectively degrades an extensive number of short-lived regulatory proteins involved in the control of normal cellular processes. In order to be degraded, proteins targeted by the ubiquitin-proteasome pathway are covalently tagged by polyubiquitination, via a three-step enzymatic process, which ultimately leads to their recognition and degradation, by the 26S proteasome in a highly specific and regulated manner. This process is accomplished by the sequential action of three enzymes: an ATP-dependent ubiquitin-activating enzyme (E1), an ubiquitin-conjugating enzyme (E2) and an ubiquitin-protein ligase (E3).\(^3\) This cascade covalently links the C terminus of ubiquitin to a free amino group on the target protein, usually the ε-amino of a lysine residue.

The 26S proteasome is comprised of a catalytic proteolytic core (20S) and an activator (19S) (Fig 2). It plays a vital role in degrading regulatory proteins that govern many signaling pathways, including the cell cycle, transcription factor activation, apoptosis, and pathways that regulate expression of proteins which direct angiogenesis, cell trafficking, and metastasis.\(^2,4\) Polyubiquinated proteins cannot be degraded directly by the active catalytic proteolytic core (20S). Rather, proteolysis requires another protein, known alternatively as PA700, ball, 19 S cap or μ-particle.\(^3\) It is 700,000 dalton, 20-subunit complex that binds to one or both of the terminal rings of the proteasome in a cooperative manner. This integral role of the 26S proteasome in cellular signal transduction has provided a new target for exploring the therapeutic potential of proteasome inhibition in neoplastic disease. It is known that several key regulatory proteins relevant to cancer initiation and progression are known to be temporally degraded during the cell cycle by the ubiquitin-proteasome pathway. Ordered ubiquitination and degradation of regulatory proteins is required for the cell to progress through the cell cycle, undergo mitosis and proliferate. Similarly, the proper function of specific ubiquitin ligases responsible for the ubiquitination of these same proteins is required for key cell cycle transitions. Aberrant degradation of cell cycle control proteins can result in accelerated and uncontrolled cell division thereby promoting cancer growth. Recent evidence from studies reveals that expression of the ubiquitin-proteasome pathway enzymes is elevated in tumor samples. The cyclin and the cyclin-dependent kinase inhibitors p21 cip1 and p27 kip1 are an example of growth regulatory proteins degraded by proteasome-dependent

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**Figure 1.** The Ubiquitin-Proteasome degradation pathway

**Proteasome Inhibitors: New Class Of Antitumor Agents**

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proteolysis. Both p21 cip1 and p27 kip1 can induce cell cycle arrest through functional inhibition of cyclin D-, E-, and A-dependent kinases. In addition, the p53 tumor suppressor required for cell cycle control and initiation of apoptosis induced by cellular damage, including ionizing radiation and chemotherapy, is also a substrate of the ubiquitin-proteasome pathway. Hence, proteasome inhibition has the potential to arrest the cell cycle in cancer cells through the disruption of a large number of growth regulatory pathways.

The ubiquitin-proteasome pathway also plays an important role in the regulation of many transcriptional responses. On the other hand, proteasome function in the cell can be regulated by altering levels of the proteasome, proteasome regulatory proteins, or proteins of the ubiquitin conjugation system.

Up-regulation of cellular proteasome levels suggests that proteolytic capacity of the proteasome per se is rate-limiting under certain conditions. This conclusion is surprising because cells normally contain a high proteasome concentration. However, changes of proteasome expression are documented in conditions associated with atrophy of skeletal muscles as a consequence of increased protein degradation. Therefore, the ubiquitin-proteasome pathway appears to be responsible for alterations in tissue size.

The relationship between proteasome function, gene transcription and potential cancer therapy is best understood for the transcription factor nuclear factor-kappa B (NF-kB) (Figure 3). NF-kB activation is regulated by 26S proteasome-mediated degradation of the inhibitor protein I-kB. NF-kB activation is integral to many aspects of tumorigenesis, such as tissue invasion and metastasis, angiogenesis, evasion of apoptosis, cell growth, and survival. Activation of NF-kB can proceed through multiple mechanisms, including autocrine or paracrine extracellular cytokine signaling, upstream oncogenic signaling mutations in NF-kB and/or I-kB, and in response to DNA damage. Cell adhesion molecules such as E-selectin, ICAM-1, and VCAM-1, as well as IL-8, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs) are regulated by NF-kB and have been implicated in tumor metastasis and angiogenesis in vivo. Furthermore, NF-kB is required in numerous cell types to maintain and control cell viability via the production of anti-apoptotic survival proteins such as cellular inhibitors of apoptosis (cIAPs), and the B-cell lymphoma-2 (Bcl-2) family of proteins. NF-kB also plays a role in cell proliferation by activating target genes of the cell cycle such as D1-cyclin, and growth factors such as interleukin-6 (IL-6). It has been demonstrated that blocking NF-kB activation by stabilizing its inhibitor, I-kB, sensitizes cells to environmental stressors and cytotoxic agents, ultimately leading to apoptosis. Hence, regulation of NF-kB dependent transcriptional regulation and activation through proteasome inhibition can impact several cancer virulence mechanisms.

Anti Tumor Activity of Proteasome Inhibitors

A number of investigators have shown that inhibitors of proteasome, including aldehydes and lactacystin, are growth inhibitory and cytotoxic for cells in culture. Boronate proteasome inhibitors have been shown to kill tumor cells in culture as demonstrated in NCI tumor cell line screen. The NCI utilizes, an in vitro screen comprised of 60 human tumor cell lines derived from 9 dif-
different cancer types (leukemia, lung, brain, colon, melanoma, ovarian, prostate, renal and breast). Data from the NCI screen showed that proteasome inhibitors have a mechanism of cytotoxicity unlike any other compound in the NCI database of 60,000 compounds. Among large number of proteasome inhibitors, PS-341 (bortezomib) was selected for intensive study based on its selectivity and chemical and biological characteristics. PS-341 specifically, selectively and reversibly inhibits the proteasome by tightly binding to the chymotrypsin-like site of the 20S core of the enzyme. By inhibiting a single molecular target, the 26S proteasome, PS-341 has the potential to affect multiple signaling pathways. The antineoplastic effects of PS-341 likely involve several distinct cell regulatory mechanisms as discussed above, including inhibition of cell growth and survival pathways, induction of apoptosis, and inhibition of gene expression integral to cellular adhesion, migration, and angiogenesis. Thus, the mechanisms by which PS-341 elicits its anti-tumor activity may vary among tumor types, and the extent to which each affected pathway is critical to the inhibition of tumor growth could also differ.

It has been demonstrated that PS-341 has a novel pattern of cytotoxicity in NCI in vitro and in vivo assays\textsuperscript{1} and displays cytotoxic activity in a variety of xenograft tumor models both as a single agent and in combination with other chemotherapeutic agents and radiation.\textsuperscript{1,15-19}

Numerous published reports show that cancer cells are more sensitive to pro-apoptotic effects of proteasome inhibition than nontransformed cells.\textsuperscript{21-27} For example, the toxicity of PS-341 for multiple myeloma (MM) cells was more than 100-fold greater when compared to peripheral blood leukocytes and normal hematopoietic cells.\textsuperscript{21,28} PS-341 has shown direct cytotoxic activity against a variety of MM cell lines and in freshly isolated cells from patients.\textsuperscript{21-22} Significantly, these studies have included myeloma cells that are highly resistant to other chemotherapeutic agents. Time-dependent exposure to PS-341 programs MM cells to commit to apoptosis.\textsuperscript{28} It was shown that this drug directly inhibits proliferation and induces apoptosis of human MM cell lines and freshly isolated patient MM cells; inhibits mitogen-activated protein kinase growth signaling in MM cells, induces apoptosis despite induction of p21 and p27 in both p53 wild-type and p53 mutant MM cells; overcomes drug resistance; adds to the anti-MM activity of dexamethasone and overcomes the resistance to apoptosis in MM cells conferred by Interleukine-6 (IL-6).\textsuperscript{28} PS-341 also inhibits the paracrine growth of human MM cells by decreasing their adherence to bone marrow stromal cells and related NF-κB-dependent induction of IL-6 secretion in bone marrow stromal cells, as well as inhibiting proliferation and growth signaling of residual adherent MM cells.

**Human Studies with PS-341**

In the fall of 1998, the first human trial with the proteasome inhibitor PS-341 was initiated at M.D.Anderson Cancer Center in Houston, Texas. Until now approximately 1500 patients were enrolled on different clinical studies employing treatment with PS-341 (Bortezomib). In May, 2003 PS-341 was approved by FDA as Velcade for Injection for the treatment of MM patients who have received at least 2 prior therapies and have demonstrated disease progression on the last therapy. Conditional approval was based mostly on the results of Phase II study of PS-341 in patients with relapsed, refractory MM (SUMMIT Trial).\textsuperscript{29} In this study 202 patients were...
enrolled and 193 could be evaluated. Most (84%) had IgG or IgA MM and advanced disease at diagnosis. Eighty percent had symptoms of peripheral neuropathy at enrollment. Of the 193 patients, 178 (92%) had previously been treated with three or more of the major classes of agents for myeloma. Patients received 1.3 mg of PS-341 per square meter of body-surface area twice weekly for 2 weeks, followed by 1 week without treatment, for up to 8 cycles (24 weeks). In patients with a suboptimal response, oral dexamethasone (20 mg daily on the day of and the day after PS-341 administration) was added to the regimen. Of the 193 patients with measurable disease, 67 (35%) had complete, partial or minimal response to PS-341 alone. Nineteen patients had complete or near-complete response. The median time to a first response was 1.3 months. The median time to progression of disease among all 202 patients while they were receiving PS-341 alone was 7 months, as compared with 3 months during the last treatment before the enrollment. According to a landmark analysis, achievement of a complete or partial response to PS-341 alone after 2 cycles was associated with significantly longer survival than that in other patients (P=0.007). Patients with a complete or partial response had significant increase in hemoglobin concentration, platelet count, levels of uninvolved immunoglobulins and performance status. The most common adverse events were gastrointestinal symptoms, fatigue, thrombocytopenia and sensory neuropathy. Drug related adverse effects led to discontinuation of PS-341 in 36 patients (18%). Ten patients (5%) died within 20 days after the last dose of PS-341, the majority of them from causes related to progressive MM. In two patients (<1%), the cause of death was assessed as possibly related to bortezomib treatment. The most clinically significant adverse event was cumulative, dose-related peripheral sensory neuropathy. Overall incidence of clinically relevant neuropathy (Grade 3) was 12 percent. However, complete resolution or improvement of peripheral neuropathy was observed in the majority of patients during the follow-up period. Second study (CREST Trial) compared two different dosages of PS-341 (1 mg/m² vs. 1.3 mg/m²) in newly diagnosed MM patients. Responses were 33% and 50%, respectively. Median time to progression was 10 and 10.9 months. Addition of dexamethasone to PS-341 in patients who failed to respond or relapsed after treatment with PS-341 alone in SUMMIT and CREST trials improved responses in 18% of patients in SUMMIT and 33% in CREST trial. An international, randomized, multicenter phase 3 trial comparing PS-341 with high-dose dexamethasone in patients with relapsed MM (APEX Study) is presently ongoing. PS-341 also showed some activity in solid tumors (prostate, lung), as well as in Non-Hodgkin Lymphoma (NHL). It will be also incorporated in high dose chemotherapy with peripheral stem cell support in MM patients. Recently PS-341 was combined with pegylated liposomal doxorubicin (Doxil) in Phase I study in relapsed/refractory MM with significant response (4/10 patients had complete or near complete response). Combination of PS-341 and Thalidomide is undergoing extensive studies, both in University of Arkansas and in large cooperative Southwestern Oncology Group. Alternative dosing schedules are investigated, including once weekly schedule. This study was initiated in Myeloma Research Program at Cleveland Clinic.

In conclusion, proteosome inhibition with PS-341 is a completely new approach to the treatment of malignancies. Development of this drug is in some way unique, since drug was originally not created as anti-malignancy drug, but showed its antitumor properties during the process of evaluation. It induces clinically significant responses in MM patients with manageable toxicity. Considering novel mode of action of this and similar drugs, future could bring promising new application in very diverse types of malignancies. New studies will provide as with clinical guidance as how to use these new agents, as well as how to prevent and manage toxicities.
References


