

Development of Proteasome Inhibitors as Therapeutic Drugs

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Abstract

The proteasome is a multi-unit enzyme complex found in the cytoplasm and nucleus of all eukaryotic cells and is responsible for degradation of unneeded or damaged intracellular proteins by proteolysis, a chemical reaction that breaks peptide bonds. Proteasome inhibition presents a promising approach to cancer therapy by targeting the proteasome function in tumor cells. Delineating the success of bortezomib in the treatment of multiple myeloma and mantle cell lymphoma, this review explores various proteasome inhibitors, currently in development, as molecular targeting agents in the fight against cancer. Proteasome inhibitors can be used alone or in combination with other conventional cancer therapies to sensitize tumor cells to cell death by various mechanisms and improve therapeutic benefits.

Keywords: Bortezomib; Proteasome inhibition; Molecular targeting drug; Cell death sensitization; Myeloma; Lymphoma; Cancer therapy

The Ubiquitin-Proteasome System

The proteasome is the main nonlysosomal endoprotease enzyme complex present in the cytoplasm and nucleus of all eukaryotic cells. It plays a critical role in the degradation of most short-lived intracellular proteins that control cellular events such as cell cycle, transcription, DNA repair, cell death, signal transduction, metabolism, morphogenesis, differentiation, antigen presentation and neuronal function. The proteasome is also responsible for protein quality control by eliminating damaged and abnormal proteins.

The proteasome is a large hollow and cylindrical 26S enzymatic complex of 2.5 MDa assembled from at least 66 proteins with the help of a number of chaperone proteins [1-3]. It is composed of the catalytic 20S core and two 19S or 11S regulatory units at either ends. The catalytic 20S core is organized into a stack of four seven-subunit rings, with the top and bottom rings formed by seven polypeptides, termed the α -subunits, and the two inner rings of seven β -subunits. The structure of the regulatory and catalytic units is illustrated in Figure 1.

Poly-ubiquitination drives the interaction between the 19S (11S) and 20S particles. It requires the activity of three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin-protein ligase (E3) [4]. The mechanisms behind polyubiquitination are still being hotly debated. In one mechanism, each Ub is added sequentially to the growing chain (termed "elongation") [5,6]. Other authors support the idea of a poly-Ub chain that is preformed and then added to the target protein [7]. Ubiquitin-receptor proteins have an N-terminal ubiquitin-like (UBL) domain, and the 19S regulatory particle has an affinity for and recognizes these UBL domains. After the poly-ubiquitinated protein is recognized by 19S, the substrate protein is unfolded by hydrolases so that it may enter the narrow gate of the 20S particle and then be degraded.

The 19S regulatory particle is divided into two subcomplexes called the base and the lid. The base consists of six AAA+ ATPases and three non-ATPase polypeptide chains [3]. The lid includes at least nine non-ATPase polypeptide chains that help remove ubiquitin from the substrates. The lid and base connection is stabilized by the Rpn10 subunit. The base of hexameric ring of 6 ATPases in the 19S regulatory particle facilitates the opening of the 20S gate and is responsible for

substrate recognition, deubiquitination, unfolding and translocation into the core particle. However, lack of structural data makes it difficult to understand how the 19S subunits are arranged and how the 19S helps with the opening of the 20S gate. The 20S catalytic core has a narrow gate at the center of the α -subunit ring where substrate proteins enter into this proteolytic degradation chamber. The gate is closed when the N-termini of the α -subunits interact, blocking substrate entry into the chamber. The 19S regulatory particle functions to help open the gate at the center of the α -subunit by interacting with this α ring and facilitate substrate access to the proteolytic chamber.

The proteolytic chamber of the 20S core has three types of catalytic activities: chymotrypsin-like, trypsin-like, and caspase-like. These activities are presented by β 5, β 2, and β 1 subunits, respectively. In immune cells the β 1, β 2, and β 5 subunits, which are constitutively expressed, are replaced by β 1i, β 2i, β 5i induced subunits to compose the immunoproteasome. Compared to the normal eukaryotic proteasome, the immunoproteasome has increased chymotrypsin-like and trypsin-like activities which assists in antigen processing [3]. Instead of the 19S regulatory particle, the immunoproteasome utilizes the 11S regulatory particle, in place of 19S, to facilitate the opening of the 20S gate and stimulates substrate unfolding and translocation. Similar to 19S, the 11S binds to the 20S core particle through the C-terminal tails of its subunits and causes α -ring conformational changes to open the proteolytic gate.

Proteasome Inhibitors

Proteasome inhibition represents a unique approach to cancer

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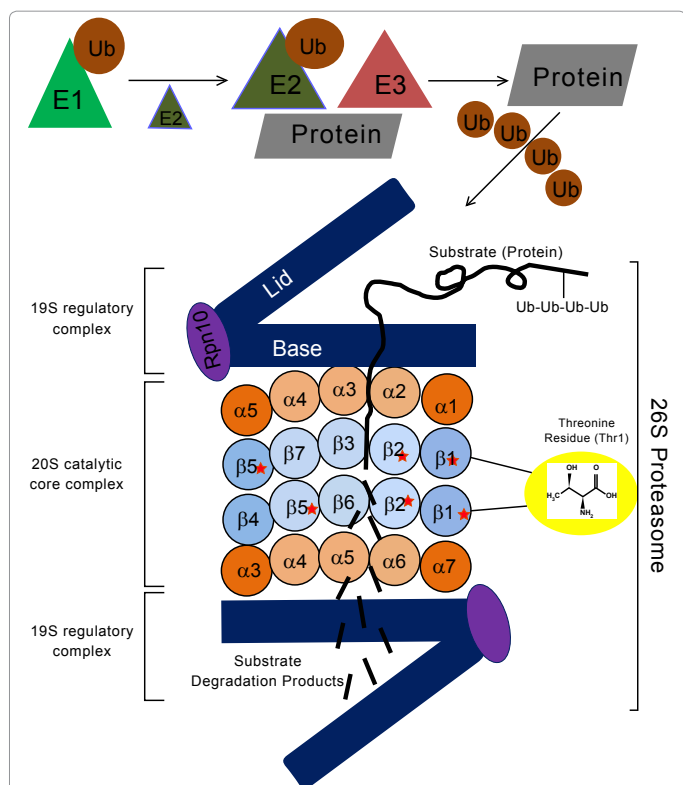


Figure 1: Ubiquitin-proteasome pathway and schematic of the cellular proteasome. The ubiquitin-proteasome pathway tags proteins with chains of ubiquitin to be degraded in the catalytic core of the proteasome. The ubiquitin E1 enzyme binds ubiquitin and transports it to the E2 enzyme. The E2 enzyme then transports the ubiquitin to the target substrate via the E3 ubiquitin ligase. Substrates (proteins) that are polyubiquitinated are degraded by the proteasome. Proteasomes regulate the concentration of particular proteins and degrade misfolded proteins. The proteasome is composed of the catalytic core 20S complex, which comprises α - and β -subunits, and two 19S regulatory complexes. Together with ATPases, these form the 26S proteasome. The degradation of the cellular proteins is a vital step in the regulation of various signal transduction pathways. The degradation process yields peptides which can then be further degraded into amino acids and used in synthesizing new proteins.

therapy by targeting the proteasome function. Following the clinical efficacy of the proteasome inhibitor bortezomib in the treatment of multiple myeloma and mantle cell lymphoma, efforts are underway to develop and test proteasome inhibitors that bind the active site of the proteasome and inhibit the complex irreversibly. Inhibitors that act on sites of the proteasome outside of the catalytic center are also in preclinical development.

Peptide aldehydes

Inhibiting proteasome function in cancer cells has been found to sensitize them to apoptotic death. Peptide aldehydes (for example, synthetic peptide MG132) were the first proteasome inhibitors. Peptide aldehydes act against serine and cysteine proteases [3]. They act on the proteasome via a nucleophilic binding. The N-terminal threonine residue at the proteasomal active site carries out a nucleophilic attack on the aldehyde moiety of the inhibitor forming a covalent hemiacetal adduct between the inhibitor and the threonine residue of the proteasome [3]. Unfortunately, peptide aldehyde inhibitors are rapidly oxidized into inactive acids in cells and transported out of the cell by the multidrug resistance (MDR) carrier system. For this reason, even though peptide aldehydes are potent inhibitors and selectively block

the chymotrypsin-like activity of the proteasome, they are not suitable candidates to be used as a therapeutic agent for the treatment of cancer.

Peptide boronates

The search for a proteasome inhibitor without the drawbacks of peptide aldehydes led to the introduction of boronic acid as a functional group. Thus, peptide boronates (for example, bortezomib- a synthetic boronate-containing inhibitor) were developed as a potential therapeutic agent to treat cancer. Boronic-acid containing inhibitors are excellent in their potency and selectively target the proteasome. Peptide boronates bind with the hydroxyl group of the N-terminal threonine residue in the proteasome by a non-covalent bond. The boron atom can receive the oxygen lone pair of the N-terminal threonine residue of the proteasome, thus forming a stable tetrahedral intermediate [4]. Unlike the peptide aldehyde, boronates are not inactivated by oxidation and are not transported from the cell by the MDR system.

Bortezomib: The peptide boronate bortezomib (N-acyl-pseudo dipeptidyl boronic acid), also known as PS-341 or Velcade™ (Millennium Pharmaceuticals), is a dipeptide that contains a boronic acid instead of a carboxylic acid at the C-terminus. Bortezomib's boronic acid reversibly binds to the chymotrypsin-like $\beta 5$ subunit of the catalytic chamber of the 20S particle and inhibits proteasome function [8]. Bortezomib's interaction with the proteasome is shown in Figure 2.

Bortezomib was an expedited product of exemplary collaboration between academia and industry. It is the first therapeutic proteasome inhibitor to be tested in humans [9]. Initial laboratory findings showed that blocking the proteasome *in vivo* did not immediately alter the normal life cycle of the cell [10]. The ensuing studies revealed that the proteasome was important in the activation of NF- κ B [11] implicated as one of the mechanisms of tumor cell resistance to apoptosis. Based

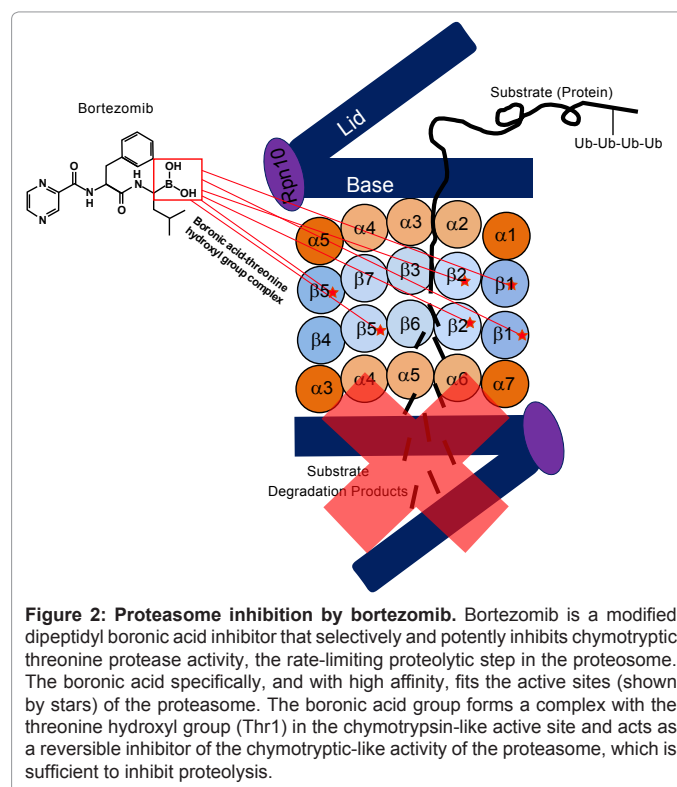


Figure 2: Proteasome inhibition by bortezomib. Bortezomib is a modified dipeptidyl boronic acid inhibitor that selectively and potently inhibits chymotryptic threonine protease activity, the rate-limiting proteolytic step in the proteasome. The boronic acid specifically, and with high affinity, fits the active sites (shown by stars) of the proteasome. The boronic acid group forms a complex with the threonine hydroxyl group (Thr1) in the chymotrypsin-like active site and acts as a reversible inhibitor of the chymotryptic-like activity of the proteasome, which is sufficient to inhibit proteolysis.

on these observations, in August 1994, Julian Adams examined bortezomib as a possible anti-cancer molecular targeting agent. By 1997, it was also shown that bortezomib could have potential influence on inhibiting tumor growth and metastasis in a mouse model of lung cancer [12]. The phase I clinical trials at the University of North Carolina presented evidence that bortezomib was effective in treating multiple myeloma. It was an exciting observation that bortezomib erased all signs of cancer from a 47-year old woman, who was diagnosed with advanced stages of multiple myeloma. Following phase II clinical trials, bortezomib was approved on May 13, 2003 by the US FDA under a Fast-Track Application, as an injectable small molecule for the treatment of multiple myeloma. In December 2006, it was approved for the treatment of relapsed or refractory mantle cell lymphoma. It remains thus far the only proteasome inhibitor approved for clinical use.

In humans, the primary route of bortezomib metabolism is by oxidative deboronation, which causes the production of two inactive diastereomeric carbinolamide metabolites. Bortezomib is excreted in the urine and bile after it has undergone secondary metabolism. Preliminary observations in myeloma patients with baseline renal insufficiency (baseline creatinine clearance of <30 ml/min) suggest that bortezomib can be given safely to patients, with comparable levels of proteasome inhibition 1 hour after dosing and comparable rates of proteasome recovery [13]. When injected intravenously in animal studies, bortezomib was observed to leave the vascular compartment within minutes. The quick metabolism of this drug makes it difficult to determine correlations between plasma concentrations and proteasome inhibition, drug toxicity, and clinical activity [13]. After the development of a pharmacodynamic assay to determine proteasome inhibition in the whole blood, it was observed that bortezomib is distributed in tissues but does not cross over to sites such as the brain, spinal cord, eyes, and testes [13].

Resistance to bortezomib: As seen with most cancer treatments, tumors can also develop resistance to bortezomib treatment. Malignant cells can either inherit or acquire mechanisms of resistance to bortezomib. Components of the proteasome can be mutated or overexpressed (such as the $\beta 5$ subunit) so that bortezomib treatment no longer inhibits the proteasomes function [14]. Levels of downstream effectors, such as increased levels of an ER homeostasis regulator and activator of UPR, chaperone protein BIP, can be altered. Heat shock proteins serve an important role in mediating resistance to apoptosis [13]. Tumor cells can develop a resistance to bortezomib by overexpressing heat shock proteins (HSP27, 70, and 90) and T cell factor 4. Tumor cells can also have constitutive NF- κ B activity that is resistant to proteasome inhibition. Malignant cells can resist by failing to accumulate pro-apoptotic proteins after bortezomib treatment, and/or increase the levels of anti-apoptotic proteins, induce autophagy, and increase the levels of anti-oxidants [14].

Novel peptide boronates

Though bortezomib treatment has shown therapeutic benefits in the clinic, detrimental side effects and resistance have been observed. Other novel proteasome inhibitors are thus in development and are undergoing clinical trials. CEP-18770, a new synthetic boronate derivative that is undergoing phase I and II trials, is an orally active proteasome inhibitor. It blocks the chymotrypsin-like activity of the proteasome at a low nanomolar concentration. MLN-9708 is another peptide boronic acid that is a reversible inhibitor of the chymotrypsin-

like subunit of the proteasome, and is also currently undergoing phase I and II trials. MLN-9708 is administered orally and is used for the treatment of solid tumors and hematological cancers [14].

Peptide epoxyketone inhibitors

Currently, two peptide epoxyketone inhibitors, Carfilzomib and ONX-0912, are being tested clinically. Peptide epoxyketone inhibitors contain an α, β -epoxyketone moiety that assists in the formation of a morpholino adduct with the N-terminal threonine residue in the proteasome, which inactivates proteasome function [4].

Carfilzomib is an irreversible inhibitor of the chymotrypsin-like subunit of the proteasome and immunoproteasome. This inhibitor has shown preclinical effectiveness against some hematological and solid malignancies both *in vitro* and *in vivo* [15]. It is administered intravenously and is currently undergoing phase II and III trials. Carfilzomib is used to treat recurrent multiple myeloma, non-Hodgkin's lymphoma and few solid tumors. Clinically, Carfilzomib has been observed to be more selective towards the chymotrypsin-like subunit than bortezomib and also overcomes resistance to conventional agents, and acts synergistically with dexamethasone to enhance cell death [4].

ONX-0912 is also an irreversible inhibitor of the chymotrypsin-like subunit of the proteasome. It is administered orally, and is currently in phase I and II trials for the treatment of solid tumors and hematological cancers.

B-lactone- γ -lactam inhibitors

The inhibitors that target the B-lactone- γ -lactam moiety are also being developed to treat malignant cells. *Marizomib* is an irreversible inhibitor of the chymotrypsin-like, caspase-like, and trypsin-like activities of the immunoproteasome. Marizomib is currently in phase Ib clinical trials and is administered intravenously. It is used to treat recurrent multiple myeloma, solid tumors, lymphomas, and leukemias [14].

Natural proteasome inhibitors

While all the inhibitors described above are synthetic, there are also natural inhibitors of the proteasome, notably, Syringolin A, TMC-95A and Argyrin A.

Syringolin A is a macrocyclic vinyl ketone inhibitor. It was isolated from the plant pathogen *Pseudomonas syringae* pv. *Syringae* [16] as a virulence factor. Similar to cells treated with proteasome inhibitors, Syringolin A was found to induce changes in gene profile expression. It inhibits proteasome function by the hydroxyl group of the catalytic threonine residue, which causes a Michael type 1,4-addition to the vinyl ketone moiety in the 14-membered ring of Syringolin A. This in turn irreversibly inhibits all three types of proteasomal activity. It was also observed that rhodamine-tagged Syringolin A selectively binds to and labels the active sites of the proteasome at therapeutic concentrations [17].

TMC-95A is a cyclic peptide inhibitor that was isolated from a fermentation broth of *Apiospora montagnei* [18,19]. TMC-95A inhibits all three types of proteasomal activity with IC_{50} values of 5.4, 200, and 60 nM, respectively. When co-crystallized with the yeast proteasome, it was observed that TMC-95A is bound to the core particle of the proteasome through specific hydrogen bonds and specifically blocks the proteasomal active site non-covalently [20,21].

Argyrin A is an immunosuppressive cyclic peptide that was isolated

originally from the myxobacterium *Archangium gephyra* [22]. It was first identified as a small molecule that promotes the accumulation of p27^{kip1}, a cyclin-dependent kinase inhibitor, in cancer cells by a high-throughput whole-cell assay. Argyrin A inhibits the chymotrypsin-like activity of the proteasome strongest, followed by a moderate inhibition of the caspase-like activity, and a weak inhibition of the trypsin-like activity [23].

Cell lineage specific inhibitor

A novel approach to proteasome inhibition may be to specifically target the hematological-specific immunoproteasome, in addition to conventional proteasome inhibitors. This specificity may increase the overall therapeutic effectiveness and reduce negative off-target effects. IPSI-001, an immunoproteasome-specific inhibitor, was shown to have inhibitory preference over the constitutive proteasome, and increase the efficiency of apoptotic induction of tumor cells from a hematologic origin [15]. IPSI-001 can be used clinically to exclusively target hematological malignancies.

Altogether, these novel proteasome inhibitor drugs are in development. They are potential molecular targeting drugs to treat cancer and may turn out to be more effective than the current FDA-approved bortezomib.

Role of Proteasome Inhibition in Tumor Cell Death Sensitization

Apoptosis sensitization

In vitro, tumor cells are found to be up to 1,000 fold more sensitive to apoptosis induced by bortezomib than normal plasma cells [24]. One reason for this response appears to be due to the ability of proteasome inhibitors to block the activation of NF- κ B, which is constitutively expressed in tumor cells [25]. The proteasome is critical for NF- κ B activation, because it catalyses both the proteolytic generation of the NF- κ B subunit p50 from the inactive p105 precursor, and the destruction of the inhibitory I κ B [11,25]. Because the proteasome is essential for NF- κ B activation, and NF- κ B also has anti-apoptotic effects; proteasome inhibitors (such as bortezomib) can sensitize tumor cells to apoptosis alone or in combination with other cancer treatments.

Amplification of extrinsic apoptosis pathway: The extrinsic pathway of apoptosis is triggered through death receptors, a member of the tumor necrosis factor (TNF) receptor superfamily. Apoptosis in response to TRAIL or TNF requires the activation of initiator caspases, which then activate the effector caspases that dismantle cells and cause death [26]. Activation of the extrinsic pathway is initiated with the ligation of these cell surface death receptors. Upon ligation with their ligands, death receptor signaling plays an important role in immune surveillance of transformed or virus-infected cells and in the removal of self-reactive lymphocytes. When a death stimulus triggers the pathway, the death ligand interacts with the inactive membrane-bound death receptor complexes and forms the death-inducing signaling complex [27]. The death-inducing signaling complex contains the adaptor protein Fas-associated death domain (FADD) protein, and caspases 8 and 10. Upon productive death-inducing signal, procaspase 8 is cleaved to convert into an active caspase 8, which in turn can activate the rest of the downstream effector caspases. In kidney cancer cells, Renca, and breast cancer cells, 4T1, bortezomib has been found to cause apoptosis sensitization by enhancing caspase 8 activity and upregulation of death receptors [28,29]. In murine Renca tumors, bortezomib also provided long term survival benefit when administered

in combination with death receptor agonist antibody [29]. Combined treatment with bortezomib and IFN- α induced synergistic apoptosis in melanoma and other solid tumor cell lines [30]. Thus, bortezomib can sensitize tumors to cell death by amplifying the extrinsic pathway of apoptosis via FADD-induced caspase-8 activation.

Amplification of intrinsic apoptosis pathway: Bortezomib can sensitize cancer cells to established cytotoxic agents and radiation therapy by targeting the intrinsic mitochondria-dependent Bcl-2 family pathway. Stimulation of the intrinsic pathway leads to the release of cytochrome-c from the mitochondria and amplification of the death signal. One of the most important regulators of this pathway is the Bcl-2 family of proteins. The Bcl-2 family members are key regulators of apoptosis and are over-expressed in many malignancies. Increased expression of Bcl-2 causes resistance to chemotherapeutic drugs and radiation therapy, while decreasing Bcl-2 expression may promote apoptotic responses to anticancer drugs [27]. The Bcl-2 family includes proapoptotic members and antiapoptotic members. Antiapoptotic Bcl-2 members act as repressors of apoptosis by blocking the release of cytochrome-c, whereas proapoptotic members act as promoters. Following a death signal, proapoptotic proteins undergo posttranslational modifications that include dephosphorylation and cleavage resulting in their activation and translocation to the mitochondria leading to apoptosis. In response to apoptotic stimuli, the outer mitochondrial membrane becomes permeable, leading to the release of cytochrome-c and second mitochondria-derived activator of caspase. Cytochrome-c, once released in the cytosol, interacts with Apaf-1, leading to the activation of caspase-9 proenzymes. Active caspase-9 then activates caspase-3, which subsequently activates the rest of the caspase cascade and leads to apoptosis. It was observed in esophageal squamous cell carcinoma (ESCC) that bortezomib treatment increased signaling via the intrinsic apoptotic pathway on subsequent exposure to TRAIL [31]. Treatment of myeloma cells with bortezomib also showed dysregulation of intracellular Ca²⁺ as a mechanism of caspase activation. Cotreatment with a panel of Ca²⁺-modulating agents identified the mitochondrial uniporter as a critical regulatory factor in bortezomib cytotoxicity [32]. Experiments with non-small-cell lung cancer (NSCLC) H460 cells showed that bortezomib treatment first leads to Bcl-2 phosphorylation and cleavage, which is accompanied by G2-M phase arrest, followed by apoptosis [33].

Bortezomib also induces the up-regulation of the pro-apoptotic protein NOXA, which interacts with the anti-apoptotic BCL-2 family proteins to induce apoptotic cell death [14]. Proteasome inhibition by bortezomib also decreases the amounts of anti-apoptotic proteins, such as MCL1, HIF1 α , and IAP and induces the accumulation of other pro-apoptotic proteins, such as p53, p27, BIM, BAX, and SMAC. Proteasome inhibition by bortezomib leads to endoplasmic reticular (ER) stress and induces the pro-apoptotic unfolded protein response. Bortezomib has been shown to induce ER stress via protein overload *in vitro* in SMILE/TMTC3 silenced HeLa cells and also increases transcript expression of a stress response protein, XBP-1, in HeLa cells and keratinocytes [34]. Other molecular mechanisms of bortezomib-induced sensitization to apoptosis include the generation of reactive oxygen species (ROS) and the induction of JUN N-terminal kinase [14].

Autophagy sensitization

Proteasome inhibitors, such as bortezomib has been shown to be a potent sensitizer to autophagic death. Autophagy is a physiological cellular process that is responsible for the degradation of intracellular material following a lack of nutrients or stress. Autophagic cell death

is characterized by massive sequestration (engulfment) of portions of the cytoplasm within autophagosomes, giving the cell a characteristic vacuolated aspect [35,36]. Once these cytoplasmic components are engulfed by the autophagosomes, they are delivered to lysosomes/vacuoles for degradation. Bortezomib promoted autophagosome formation, stimulated autophagic flux, and upregulated expression of the autophagy-specific genes ATG5 and ATG7 in some human prostate cancer cells and immortalized mouse embryonic fibroblasts (MEFs) [37]. Upregulation of ATG5 and ATG7 only occurred in cells displaying proteasome inhibitor-induced phosphorylation of the eukaryotic translation initiation factor 2 alpha (eIF2 α). These data collectively showed that proteasome inhibition activates autophagy through a phosphor-eIF2 α -dependent mechanism to eliminate protein aggregates and alleviate proteotoxic stress.

The autophagic marker LC3 (microtubule-associated protein1 light chain 3) is a reliable marker of autophagosomes, and the conversion of LC3-I to LC3-II is indicative of autophagic activity. After human endothelial cells were treated with bortezomib, intense cytoplasmic vacuolization with the evidence of autophagosomes at electron microscopy, and conversion toward the autophagosome-associated LC3-II form was observed [38]. The authors also found that after the removal of bortezomib, there is a recovery of cell shape and viability. These findings suggested that bortezomib can induce autophagy in endothelial cells.

Conclusions and Future Perspective

Only a few cancers have been identified so far to be susceptible to bortezomib treatment alone or in combination with standard anti-cancer therapies. The novel proteasome inhibitors in development that can block the catalytic active site of the proteasome more selectively and potently in tumor cells may increase therapeutic efficacy following standard chemotherapy and radiation therapy. The new inhibitors may even decrease resistance to chemotherapies and irradiation shown by tumors. More research must be conducted to fully understand the therapeutic range and safety of these novel proteasome inhibitors, and their effect on host immune responses against cancer.

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References

1. Ciechanover A (2005) Proteolysis: from the lysosome to ubiquitin and the proteasome. *Nat Rev Mol Cell Biol* 6: 79-87.
2. Hershko A, Ciechanover A (1998) The ubiquitin system. *Annu Rev Biochem* 67: 425-479.
3. Xie Y (2010) Structure, assembly and homeostatic regulation of the 26S proteasome. *J Mol Cell Biol* 2: 308-317.
4. Tsukamoto S, Yokosawa H (2010) Inhibition of the ubiquitin-proteasome system by natural products for cancer therapy. *Planta Med* 76: 1064-1074.
5. Li W, Tu D, Brunger AT, Ye Y (2007) A ubiquitin ligase transfers preformed polyubiquitin chains from a conjugating enzyme to a substrate. *Nature* 446: 333-337.
6. Ravid T, Hochstrasser M (2007) Autoregulation of an E2 enzyme by ubiquitin-chain assembly on its catalytic residue. *Nat Cell Biol* 9: 422-427.
7. Jin L, Williamson A, Banerjee S, Philipp I, Rape M (2008) Mechanism of ubiquitin-chain formation by the human anaphase-promoting complex. *Cell* 133: 653-665.
8. Ruschak AM, Slassi M, Kay LE, Schimmer AD (2011) Novel proteasome inhibitors to overcome bortezomib resistance. *J Natl Cancer Inst* 103: 1007-1017.
9. Sanchez-Serrano I (2006) Success in translational research: lessons from the development of bortezomib. *Nat Rev Drug Discov* 5: 107-114.
10. Goldberg A (2004) Cancer Drug Discovery and Development: Proteasome Inhibitors in Cancer. 17-38.
11. Palombella VJ, Rando OJ, Goldberg AL, Maniatis T (1994) The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 78: 773-785.
12. Teicher BA, Ara G, Herbst R, Palombella VJ, Adams J (1999) The proteasome inhibitor PS-341 in cancer therapy. *Clin Cancer Res* 5: 2638-2645.
13. Voorhees PM, Orlowski RZ (2006) The proteasome and proteasome inhibitors in cancer therapy. *Annu Rev Pharmacol Toxicol* 46: 189-213.
14. Wang F, Zhai S, Liu X, Li L, Wu S, et al. (2011) A novel dithiocarbamate analogue with potentially decreased ALDH inhibition has copper-dependent proteasome-inhibitory and apoptosis-inducing activity in human breast cancer cells. *Cancer Lett* 300: 87-95.
15. Kuhn JD, Orlowski ZR, Bjorklund CC (2011) Second generation proteasome inhibitors: carfilzomib and immunoproteasome-specific inhibitors (IPSI). *Curr Cancer Drug Targets* 11: 285-95.
16. Groll M, Schellenberg B, Bachmann AS, Archer CR, Huber R, et al. (2008) A plant pathogen virulence factor inhibits the eukaryotic proteasome by a novel mechanism. *Nature* 452: 755-758.
17. Clerc J, Florea BI, Kraus M, Groll M, Huber R, et al. (2009) Syringolin A selectively labels the 20S proteasome in murine EL4 and wild-type and bortezomib-adapted leukaemic cell lines. *ChemBioChem* 10: 2683-2643.
18. Koguchi Y, Kohno J, Nishio M, Takahashi K, Okuda T, et al. (2000) TMC-95A, B, C, and D, novel proteasome inhibitors produced by *Apiospora montagnei* Sacc. *TC* 1093. *J Antibiot* 53: 105-109.
19. Kohno J, Koguchi Y, Nishio M, Nakao K, Kuroda M, et al. (2000) Structures of TMC-95A-D: novel proteasome inhibitors from *Apiospora montagnei* Sacc. *TC* 1093. *J Org Chem* 65: 990-995.
20. Groll M, Koguchi Y, Huber R, Kohno J (2001) Crystal structure of the 20S proteasome: TMC-95A complex: a non-covalent proteasome inhibitor. *J Mol Biol* 311: 543-548.
21. Groll M, Götz M, Kaiser M, Weyher E, Moroder L (2006) TMC-95-based inhibitor design provides evidence for the catalytic versatility of the proteasome. *Chem Biol* 13: 607-614.
22. Sasse F, Steinmetz H, Schupp T, Petersen F, Memmert K (2002) Argynins, immunosuppressive cyclic peptides from myxobacteria. I. Production, isolation, physico-chemical and biological properties. *J Antibiot* 55: 543-551.
23. Nickeleit I, Zender S, Sasse F, Geffers R, Brandes G (2008) Argynin A reveals a critical role for the tumor suppressor protein p27^{kip1} in mediating antitumor activities in response to proteasome inhibition. *Cancer Cell* 14: 23-25.
24. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, et al. (2001) The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res* 61: 3071-3076.
25. Barnes PJ, Karin M (1997) Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 336: 1066-1071.
26. Albeck JG, Burke JM, Aldridge BB, Zhang M, Lauffenburger DA (2008) Quantitative analysis of pathways controlling extrinsic apoptosis in single cells. *Mol Cell* 30: 11-25.
27. Ghobrial IM, Witzig TE, Adjei AA (2005) Targeting Apoptosis Pathways in Cancer Therapy. *CA Cancer J Clin* 55: 178-194.
28. Brooks AD, Jacobsen KM, Li W, Shanker A, Sayers TJ (2010) Bortezomib sensitizes human renal cell carcinomas to TRAIL apoptosis through increased activation of caspase-8 in the death-inducing signaling complex. *Mol Cancer Res* 8: 729-738.
29. Shanker A, Brooks AD, Tristan CA, Wine JW, Elliott PJ, et al. (2008) Treating

- metastatic solid tumors with bortezomib and a tumor necrosis factor-related apoptosis-inducing ligand receptor agonist antibody. *J Natl Cancer Inst* 100: 649-662.
30. Lesinski GB, Raig ET, Guenterberg K, Brown L, Go MR (2008) IFN-alpha and bortezomib overcome Bcl-2 and Mcl-1 overexpression in melanoma cells by stimulating the extrinsic pathway of apoptosis. *Cancer Res* 68: 8351-8360.
31. Seki N, Toh U, Sayers TJ, Fujii T, Miyagi M, et al. (2010) Bortezomib sensitizes human esophageal squamous cell carcinoma cells to TRAIL-mediated apoptosis via activation of both extrinsic and intrinsic apoptosis pathways. *Mol Cancer Ther* 9: 1842-1851.
32. Landowski TH, Megli CJ, Nullmeyer KD, Lynch RM, Dorr RT (2005) Mitochondrial-mediated dysregulation of Ca²⁺ is a critical determinant of Velcade (PS-341/bortezomib) cytotoxicity in myeloma cell lines. *Cancer Res* 65: 3828-3836.
33. Ling YH, Liebes L, Ng B, Buckley M, Elliott PJ (2002) PS-341, a novel proteasome inhibitor, induces Bcl-2 phosphorylation and cleavage in association with G2-M phase arrest and apoptosis. *Mol Cancer Ther* 1: 841-849.
34. Racape M, Duong Van Huyen JP, Danger R, Giral M, Bleicher F, et al. (2011) The involvement of SMILE/TMTC3 in endoplasmic reticulum stress response. *PLoS One* 6: e19321.
35. Shintani T, Klionsky DJ (2004) Autophagy in health and disease: a double-edged sword. *Science* 306: 990-995.
36. Tsujimoto Y, Shimizu S (2005) Another way to die: autophagic programmed cell death. *Cell Death Differ* 2: 1528-1534.
37. Zhu K, Dunner K Jr, Mcconkey DJ (2010) Proteasome inhibitors activate autophagy as a cytoprotective response in human prostate cancer cells. *Oncogene* 29: 451-462.
38. Belloni D, Veschini L, Foglieni C, Dell'Antonio G, Caligaris-Cappio F (2010) Bortezomib induces autophagic death in proliferating human endothelial cells. *Exp Cell Res* 316: 1010-1018.