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CANCER

Proteasome inhibition and cancer therapy

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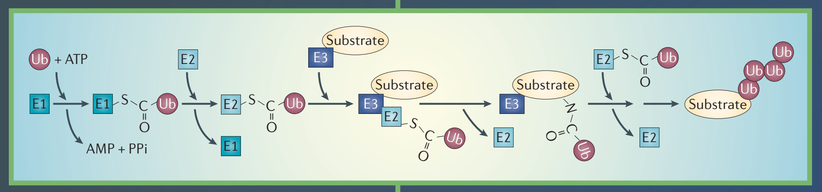
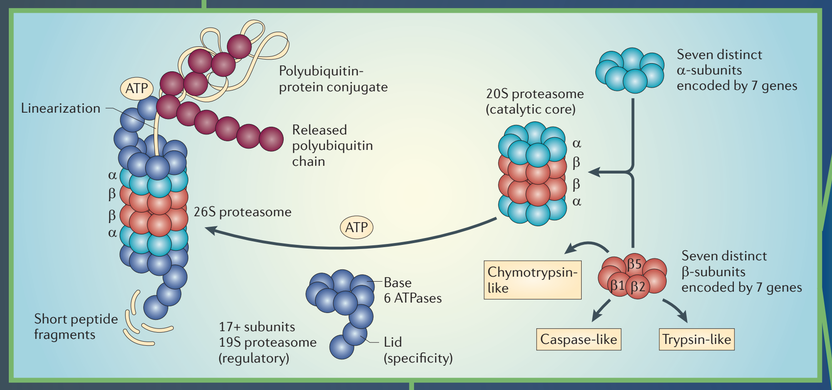
The approval of the proteasome inhibitor bortezomib by the US Food and Drug Administration (FDA), for the treatment of malignant diseases, either alone or in combination with other therapies, represents an important milestone in proteasome-targeted cancer therapy. Although bortezomib treatment results in clinical benefit, substantial side effects and resistance have been observed. Therefore, other novel proteasome inhibitors, such as carfilzomib, marizomib, immunoproteasome inhibitors (IPSIs) and several natural products are being tested in clinical trials. Furthermore, copper-binding drugs, such as clioquinol and disulfiram, can also inhibit proteasome activity in human cancer cells. This poster shows some of these recent exciting discoveries and identifies some of the areas where targeting the ubiquitin–proteasome pathway has particular promise for the future of cancer treatment.

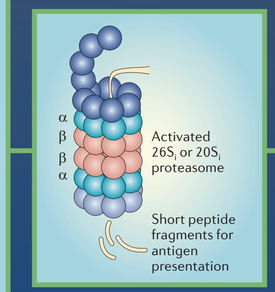


The ubiquitin (Ub)-conjugating system is characterized by three distinct enzymes, Ub-activating (E1), Ub-conjugating (E2) and Ub-ligating (E3) enzymes, that function in linking ubiquitin molecules to protein substrates through covalent modification in a sequential multistep process. Because the ubiquitin-conjugating system plays such a crucial part in the regulation of protein turnover in the cell and is located upstream of the proteasome complex, various inhibitors have been developed that target the E1, E2 and especially E3 enzymes, as well as the ubiquitin receptors and deubiquitylating enzymes.



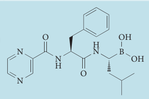
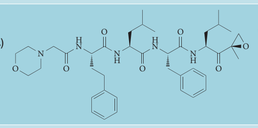
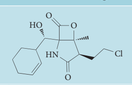
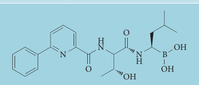
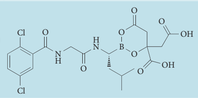
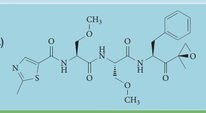
The 26S proteasome is a multisubunit protease complex that is localized in both the nucleus and the cytosol of eukaryotic cells and that selectively degrades not only redundant or damaged proteins but also many regulatory proteins. The 26S proteasome complex is comprised of the 20S proteasome, which serves as the catalytic core, and two 19S regulatory units at either end. The α-subunits guard the entrance to the active sites of the β-subunits by restricting the access to unfolded proteins only. The β1, β2 and β5 subunits are responsible for the proteolytic activities. In all three β-subunits, a Thr1 residue is responsible for catalysis, which is accomplished through nucleophilic attack. At a molecular level, bortezomib predominantly forms a reversible covalent complex with the proteasomal β5 active site (β5> β1>> β2), although it binds to all three catalytic β-subunits in an essentially identical manner: the oxygen atom on the side chain of the amino-terminal Thr1 of each subunit binds to the boron atom in bortezomib owing to its Lewis acidity and forms a tetrahedral adduct. The 19S regulatory unit consists of six ATPase and at least eight non-ATPase subunits that are required for protein recognition, deubiquitylation, substrate unfolding and translocation for access to the 20S catalytic core.

The immunoproteasome ($20S_i$ and $26S_i$), an interferon-γ-inducible form of the proteasome, is derived from replacement of the β1, β2 and β5 subunits with the immunoproteasome-specific $β1_i$, $β2_i$ and $β5_i$ subunits, respectively. This results in modified substrate specificity, altered proteolytic activity and a differential response to inhibitors. Increased expression of the immunoproteasome complex has been reported in multiple myeloma. IPSI-001 has been shown to preferentially inhibit immunoproteasome $20S_i$ activity (mainly by binding to the $β1_i$ subunit) resulting in increased apoptotic cell death in human haematopoietic cancer cells. Other IPSIs that are being developed include PR-924, PR-957 and other $β1_i$-specific inhibitors.

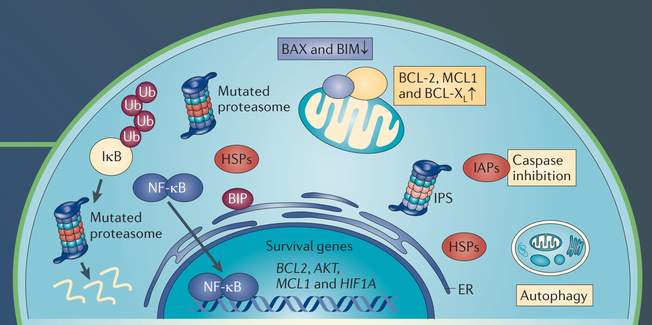


Although bortezomib has been used successfully in treating haematological malignancies (specifically multiple myeloma and mantle cell lymphoma), its effect on solid tumours has been less promising. This lack of efficacy in solid tumours may be due to the nature of reversible proteasome inhibition by bortezomib; its short duration of activity; and inherent or acquired resistance in tumour cells. The clinical use of bortezomib is also hampered by dose-limiting toxicities, such as peripheral neuropathy.

Table 1 | **Proteasome inhibitors as anticancer drugs in clinical development**

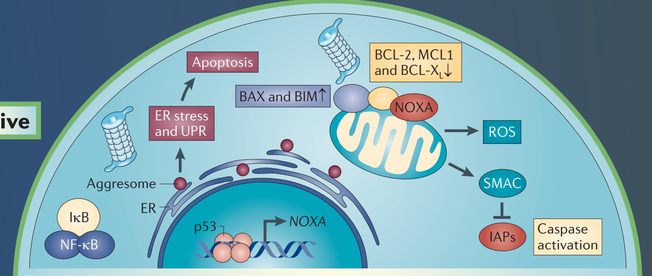
Proteasome inhibitor (developer)	Chemical structure	Structural class	Inhibition type	Inhibition profile	Development status	Types of cancers treated	Route of administration
Bortezomib (Millennium Pharmaceuticals)		Peptide boronic acid	Reversible	CT-L, C-L and immuno-proteasome	Approved	Multiple myeloma, recurrent multiple myeloma and mantle cell lymphoma	Intravenous
Carfilzomib (Onyx Pharmaceuticals)		Peptide epoxyketone	Irreversible	CT-L and immuno-proteasome	Phase II–III	Recurrent multiple myeloma, non-Hodgkin's lymphoma and solid tumours	Intravenous
Marizomib (Nereus)		β-lactone-γ-lactam	Irreversible	CT-L, C-L, T-L and immuno-proteasome	Phase Ib	Recurrent multiple myeloma, solid tumours, lymphomas and leukaemias	Intravenous
CEP-18770 (Cephalon)		Peptide boronic acid	Reversible	CT-L	Phase I–II	Reccurrent multiple myeloma, advanced stage solid tumours, lymphoblastic leukaemia and non-Hodgkin's lymphoma	Intravenous or oral
MLN-9708 (Millennium Pharmaceuticals)		Peptide boronic acid	Reversible	CT-L	Phase I and II	Lymphoma and solid tumours	Intravenous or oral
ONX-0912 (Onyx Pharmaceuticals)		Peptide epoxyketone	Irreversible	CT-L	Phase I–II	Solid tumours and haematological cancers	Oral

Resistant



Mechanisms of bortezomib resistance, which can be either inherent or acquired, include: mutation or overexpression of components of the proteasome (such as the β5 subunit); alteration in levels of downstream effectors, such as increased levels of chaperone protein BIP (a central regulator of ER homeostasis and activation of UPR); overexpression of heat shock proteins (HSP27, HSP70 and HSP90) and T cell factor 4; constitutive NF-κB activity that is resistant to proteasome inhibition; failure to accumulate pro-apoptotic proteins after proteasome inhibition; increased levels of anti-apoptotic proteins; induction of autophagy; and high levels of anti-oxidants.

Sensitive



The NF-κB inhibitory protein IκB is normally degraded by the ubiquitin–proteasome pathway and so accumulates when the proteasome is inhibited, resulting in the down-regulation of anti-apoptotic NF-κB-target genes. Bortezomib also induces the upregulation of NOXA, a pro-apoptotic protein that interacts with anti-apoptotic proteins of the BCL-2 family, such as BCL-X_L and BCL-2, resulting in apoptotic cell death in malignant cells. Proteasome inhibition also leads to the accumulation of other pro-apoptotic proteins, including p53, p27, BAX, BIM and SMAC (also known as Diablo) and decreased levels of some anti-apoptotic proteins, such as MCL1, IAP and HIF1α. A major effect of proteasome inhibition by bortezomib is induction of ER stress and triggering of the pro-apoptotic unfolded protein response. Other molecular mechanisms of action include: the generation of reactive oxygen species (ROS) and the induction of JUN N-terminal kinase.

Onyx Pharmaceuticals

Onyx Pharmaceuticals, Inc. is a global biopharmaceutical company engaged in the development and commercialization of innovative therapies for improving the lives of people with cancer and other serious diseases. The company is focused on developing novel medicines that target key molecular pathways, to transform the treatment of life-threatening diseases.

Onyx has established a development pipeline of anticancer compounds at various stages of clinical testing, including carfilzomib, a selective next-generation proteasome inhibitor that is currently being evaluated in multiple clinical trials for the potential treatment of patients with multiple myeloma. In clinical studies, carfilzomib has demonstrated encouraging activity across a range of treatment settings and patient populations. Based on complete results from a Phase 2b study of single-agent carfilzomib in patients with relapsed and refractory multiple myeloma, Onyx has filed a new drug application (NDA) with the U.S. Food and Drug Administration for accelerated approval.

In addition to carfilzomib, Onyx is developing two other novel proteasome inhibitors, including an oral protease inhibitor (ONX 0912) and an immunoproteasome inhibitor (ONX 0914). The proteasome has been validated as an important clinical target in cancer, and Onyx's goal is to develop next-generation agents with high degrees of specificity that provide potential increased therapeutic efficacy and reduced off-target toxicities.

Onyx today has three major areas of focus: an approved tyrosine kinase inhibitor, an emerging proteasome inhibitor franchise, and an earlier stage pipeline. The company is committed to advancing innovative compounds, with the goal of extending and enhancing the lives of patients with life-threatening diseases. For more information about Onyx, visit the company's website at www.onyx-pharm.com.

Abbreviations

C-L, caspase-like; CT-L, chymotrypsin-like; ER, endoplasmic reticulum; HIF1α, hypoxia-inducible factor 1α; HSPs, heat shock proteins; IAP, inhibitor of apoptosis; IκB, inhibitor of NF-κB; NF-κB, nuclear factor-κB; T-L, trypsin-like; UPR, unfolded protein response.

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Acknowledgements

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