Published in final edited form as:
Semin Hematol. 2012 July ; 49(3): . doi:10.1053/j.seminhematol.2012.04.003.

The Immunoproteasome as a Target in Hematologic Malignancies

Deborah J. Kuhn[1] and **Robert Z. Orlowski**[1,2]

[1]The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma and Myeloma, Houston, TX

[2]Department of Experimental Therapeutics, Division of Cancer Medicine, Houston, TX

Abstract

Suppression of proteasome function with the first-in-class small molecule inhibitor bortezomib is a rational therapeutic strategy against several hematologic malignancies, including multiple myeloma and mantle cell lymphoma. Second generation inhibitors such as carfilzomib, ixazomib, and marizomib that, like bortezomib, target both the constitutive proteasome and the immunoproteasome, are also in clinical trials and showing encouraging activity. While the efficacy of these agents is well documented, toxicities associated with their use, such as peripheral neuropathy and gastrointestinal effects, can necessitate dose reductions or even discontinuations, possibly hampering their anti-neoplastic effects. These findings suggested that it could be possible to improve the therapeutic index of this class of drugs by specifically targeting only the immunoproteasome. Since the immunoproteasome is a unique target found in lymphoid-derived cells, immunoproteasome-specific inhibitors (IPSIs) could preserve efficacy while reducing treatment-emergent toxicities since they would spare other tissues with little to no immunoproteasome expression. This review discusses the current state of development of IPSIs, and the potential of using such agents for the treatment of hematologic malignancies.

Introduction

One focus of translational research is to identify novel drugs that may be able to preferentially target malignant cell populations and to some extent spare normal cells, since traditional cytotoxic drugs can induce toxicities that limit their therapeutic utility. Probably one of the best known targeted small molecule therapeutics of this type is imatinib mesylate (Gleevec™; Novartis; East Hanover, New Jersey). This drug suppresses the function of the Bcr-Abl tyrosine kinase that is produced by the Philadelphia chromosome translocation, and has revolutionized the care of patients with chronic myelogenous leukemia.[1,2] Another agent that has had a huge impact on clinical practice in the hematologic malignancies is the proteasome inhibitor bortezomib (Velcade™; Millennium: The Takeda Oncology Company; Cambridge, Massachusetts).[3–5] While bortezomib inhibits proteasomes found in all cell

Address correspondence to: Dr. Robert Z. Orlowski, The University of Texas M. D. Anderson Cancer Center, Department of Lymphoma & Myeloma, 1515 Holcombe Blvd., Unit 429, Houston, TX 77030-4009, rorlowsk@mdanderson.org, Telephone 713-794-3234, Fax 713-563-5067.

Disclosures: R.Z.O. serves on advisory boards for Millennium: The Takeda Oncology Company and Onyx Pharmaceuticals.

types, it is targeted in the sense that malignant plasma cells seem particularly sensitive to its effects, possibly due to their high protein synthetic capacity. The latter places a higher load of poorly folded proteins onto the ubiquitin-proteasome pathway (UPP) at baseline, which may compromise its capacity to remove any additional toxic proteins of the type that accumulate during proteasome inhibition.[6]

Proteasomes are large, multi-subunit complexes located in both the cytosol and nucleus that are responsible for degrading the majority of intracellular proteins as part of the UPP. The function of the UPP proteolytic machinery also extends beyond the degradation of obsolete proteins, and is also critical for a number of cellular processes including proliferation, inflammation, stress response, antigen presentation, and apoptosis.[7] Most cells in eukaryotes express subunits that are assembled into the constitutive proteasome, which contains a 20S core particle with at least three different proteolytic activities. It is composed of four stacked rings of α and β subunits, with each ring containing seven unique subunits in an α-β-β-α configuration.[8,9] The two β subunit rings form the inner portion of the proteasome with proteolytic activity, while the two α subunit rings are on the outside of the barrel-shaped complex and provide structural integrity to the proteasome, and participate in substrate recognition with additional cap structures. Three main catalytic activities are associated with the proteasome, including the chymotrypsin-like (ChT-L, found in the β5 subunit), trypsin-like (T-L, in the β2 subunit), and the caspase-like (C-L, found in the β1 subunit) activities.[10,11]

A second proteasome variant is known as the immunoproteasome, a term that arises from the fact that it was discovered during studies of antigen presentation on the cell surface for T-cell recognition to stimulate immune response in collaboration with major histocompatibility class I (MHC class I) molecules. The immunoproteasome, or $20S_i$, differs from the 20S constitutive proteasome in that the proteolytic β5, β2, and β1 subunits are replaced with different subunits known as $\beta5_i$ (LMP-7), $\beta2_i$ (LMP-10, MECL1), and $\beta1_i$ (LMP-2). Immunoproteasome expression levels are commonly found to be elevated in cells of hematopoietic origin[12–14]. The $20S_i$ displays a higher affinity for peptidic cleavage following hydrophobic and basic residues, indicating an enhanced ChT-L ($\beta5_i$) and T-L ($\beta2_i$) activity, and a reduction in the C-L ($\beta1_i$) activity compared to the constitutive 20S.[15] This altered proteolytic activity results in the production of peptide substrates that are highly optimized for presentation to MHC class I molecules.[16,17] Preferential assembly of $20S_i$ immunoproteasomes over any constitutive 20S proteasomes will occur when the $\beta5_i$ subunit is present.[18] Additionally, stimulation with type I interferon[19], tumor necrosis factor (TNF)-α[20], or interferon γ[21], cytokines essential for both innate and adaptive immunity to viral and bacterial infection, will also stimulate new $20S_i$ biogenesis. The fact that these cytokines and $\beta5_i$ expression stimulate $20S_i$ assembly is an indication of the critical function of the inducible immunoproteasome in the face of infection. However, while these characteristics directly associate the $20S_i$ with antigen presentation, the functions of either the 20S or $20S_i$ are not discrete, as the 20S is also capable of processing substrates for MHC presentation[22], and conversely, the $20S_i$ may also manage common constitutive targets.[13,23] In addition to the 20S and $20S_i$ catalytic cores, the proteasome also can be capped at either one or both ends with regulators called 19S (20S) or PA28 ($20S_i$). These regulators enhance proteolytic cleavage of substrates in a ubiquitin-and ATP-dependent manner for the 19S (PA700)[24,25], and in a ubiquitin- and ATP-independent manner for PA28 (11S)[26], respectively.

IPSIs in Hematologic Malignancies

IPSI-001

Considerable interest has been focused on developing immunoproteasome-specific inhibitors (IPSIs) for applications in immunology since they may combat disorders such as

systemic lupus erythematosus[27], inflammatory bowel disease[28], and rheumatoid arthritis[29]. Distribution of the $20S_i$ proteasome is generally limited to the spleen, thymus, bone marrow, and lymph nodes, all of which are associated with lymphocyte maturation. The liver, intestine, and lungs also have $20S_i$ expression, likely due to the need for active immune function due to their contact with ingestible and inhaled foreign matter.[13,30] Currently, bortezomib is the only proteasome inhibitor approved by the Food and Drug Administration for the treatment of multiple myeloma and mantle cell lymphoma. Unfortunately, grade three or four toxicities sometimes require dose reductions in this chemotherapeutic, or even its discontinuation. Peripheral neuropathy in particular is found in up to 40% or more of patients treated with bortezomib, and has recently been linked to a secondary target of bortezomib, HtrA2/Omi, a stress induced protease important for neuronal cell survival and neurite formation.[31] Given the relatively limited expression of $20S_i$ in cells of lymphoid origin, ISPIs could be used to specifically target hematologic malignancies that originate from lymphoid cells while sparing neural and other tissues.

Early studies attempting to develop tumor targeting ISPIs were undertaken by identifying immunoproteasome specific inhibitors using an *in vitro* screen of potential peptides against purified proteasome preparations from bovine pituitaries (20S) and splenocytes ($20S_i$).[32] Seven rationally designed peptidyl-aldehyde inhibitors synthesized through oxidation of the corresponding peptidyl alcohols were screened.[33,34] One out of the seven inhibitors, carbobenzoxy-leucyl-norleucinal (Z-LnL-CHO, or IPSI-001), also known as calpeptin, displayed a K_i that was 100-fold lower for the inhibition of the ChT-L activity of the $20S_i$ as compared to the constitutive 20S proteasome.[32] IPSI-001 also retained its preferential $20S_i$ activity in cytotoxicity assays, with cell lines representing hematologic cancers being more sensitive to IPSI-001-induced cell death than a panel of solid tumor cell lines that were tested.[35] Notably, the sensitivity of cells to these cytotoxic effects correlated well with the expression levels of immunoproteasome subunits. Similar to bortezomib and carfilzomib, IPSI-001 induced apoptosis that involved both intrinsic (caspase 9-mediated) and extrinsic (caspase 8-mediated) cell death pathways, which then converged upon the common effector, caspase-3. Other consequences of IPSI-001 treatment included cleavage of poly(ADP)-ribose polymerase (PARP) and depolarization of the trans-mitochondrial membrane potential. IPSI-001 also displayed potent activity against human multiple myeloma cell lines that were resistant to doxorubicin, melphalan, and bortezomib.[32] Importantly, IPSI-001 was effective against patient-derived samples of acute myeloid leukemia, chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin's lymphoma, as well as against purified $CD138^+$ plasma cells from patients with clinical resistance to bortezomib.[32]

An additional interesting observation regarding IPSI-001 was that it bound to the $\beta1_i$ subunit (C-L activity) in both purified proteasomes, and in whole cell extracts, with no obvious binding to either the $\beta2_i$ or $\beta5_i$ subunits, or to any portion of the constitutive 20S. However, examination of the proteolytic inhibitory effects of IPSI-001 showed that the potency was greatest against the ChT-L activity, with an order of ChT-L > C-L >> T-L. Notably, Ho et al. showed that a $\beta1_i$ specific inhibitor developed by their group also had a considerable ability to inhibit the ChT-L activity[36], and Kisselev et al. found a highly specific β1 inhibitor that displayed both ChT-L and C-L inhibition.[37] There are several possible explanations, postulated by Kisselev et al.[37], for an inhibitor binding to $\beta1_i$ and yet exerting its effects primarily on $\beta5_i$ activity: (1) direct inhibition of C-L activity facilitated through specific β1 binding; (2) non-catalytic regulatory site binding of the β1 inhibitor resulting in indirect inhibition of the ChT-L protease activity; and finally, (3) β1 inhibitor allosteric inhibition of the ChT-L activity. Considering the results obtained using IPSI-001 and those generated by Ho *et al*. with another $\beta1_i$ specific inhibitor, it was felt that the last of these models would most likely account for the observed effects of IPSI-001. Moreover, the data support the

hypothesis that the ChT-L activity must be inhibited at a minimum to achieve a pro-apoptotic response.[32]

PR-924

Many proteasome inhibitors like bortezomib actually target the $20S_i$ at subnanomolar concentrations over the nanomolar concentrations necessary for 20S inhibition. To identify selective inhibitors of both the $20S_i$ and 20S to help discern the specific effects of inhibiting only one proteasome variant, Leonie et al. focused on peptide epoxy-ketone derivatives related to carfilzomib (Onyx Pharmaceuticals, Inc.; South San Francisco, CA), a novel, irreversible tetra-peptide ketoepoxide currently in phase III clinical trials for myeloma. Compared to the non-specific effects of carfilzomib, they identified PR-924, a tri-peptide ketoepoxide, which showed 130-fold selectivity for $\beta5_i$ over the constitutive β5 subunit.[14] Interestingly, they found that pan-proteasome inhibition with carfilzomib, or with PR-924 in combination with another peptide epoxy-ketone with specificity for the 20S β5 subunit, was capable of inducing a cytotoxic effect in malignant and normal, non-transformed cells. In contrast, inhibition of $\beta5_i$ alone, or of β5 alone, did not induce apoptosis of either normal or neoplastic cells. These data suggest that, in cells which express both the constitutive and immunoproteasome, dual inhibition of both β5 and $\beta5_i$ with either non-specific agents, or with a combination of IPSIs and constitutive proteasome-specific inhibitors (CPSIs) is needed for anti-tumor activity.

The molecular sequelae of specific inhibition with CPSI or PR-924 were examined as well by Leonie et al., who found that treatment with either inhibitor alone was not sufficient to induce accumulation of ubiquitinated proteins, or of Noxa or IκBα.[14] Only when the inhibitors were combined for pan-proteasome inhibition was accumulation observed, which mirrored the levels observed in carfilzomib treated cells. A characteristic of proteasome inhibition is also the induction of C/EBP homologous protein (CHOP), an endoplasmic reticulum stress molecule that is increased as a consequence of the ubiquitinated protein aggregates formed in the cell after proteasome inhibition. PR-924 alone was insufficient to induce CHOP expression, as was treatment with a CPSI, while pan-proteasome inhibition with both agents simultaneously led to induction of CHOP expression.[14]

Leonie et al. also reported the relative levels of proteasome subunit expression in both tumor cell lines and in primary cells from patients. They found heterogeneity in immunoproteasome subunit expression in cell lines of hematologic origin ranging from 37% of all proteasome content to 69%. Plasma cell samples from healthy donors, newly diagnosed myeloma, refractory myeloma, and peripheral blood mononuclear cells displayed a generally greater percentage of $20S_i$ subunits, ranging from 60% to 97%. These data suggest that targeting either the β5 or $\beta5_i$ subunit alone was not efficacious in the malignant hematologic cell lines because of their mixed expression of proteasome variants. They also indicate that there is a redundancy in proteasome function, since abrogation of constitutive proteasome activity or immunoproteasome activity alone was incapable of inducing cell death and/or the characteristic molecular sequelae of generalized proteasome inhibition.[14] However, the homogeneity of $20S_i$ expression in cells of hematopoietic origin from patients indicates that IPSIs still hold promise as potent, efficacious agents in the clinic for patients with hematologic malignancies.

Further data presenting the promise of targeting the immunoproteasome were published by Singh et al., who examined the effect of PR-924 on multiple myeloma cell growth *in vitro* and *in vivo*.[38] Contrary to the findings of Parlati et al.[14], they found that 3/4 multiple myeloma cell lines had >70% immunoproteasome expression, and only 1/3 $CD138^+$ plasma cell isolates had >70% $20S_i$. They also tested the possibility that PR-924 was capable of inducing cell death in a number of human myeloma cell lines, including those with

resistance to doxorubicin and dexamethasone, and found that PR-924 potently induced apoptosis. PR-924 also activated cell death in patient samples which was independent of the cytoprotective effect of bone marrow stromal cells or interleukin (IL)-6 supplementation, and had minimal activity on five peripheral blood mononuclear cell samples.[38] Singh et al. also found that PR-924 was effective in suppressing tumor growth by 2.3-fold compared to vehicle controls in two distinct plasmacytoma xenograft mouse models. A 3.4-fold reduction in soluble human IL-6 receptor (shuIL6R) levels was seen, which typically correlate well with tumor reduction, and the mice tolerated the treatment well over a three-week course.

ONX0914 (PR-957)

ONX0914 is another epoxyketone derivative related to carfilzomib that provides selective inhibition of $\beta5_i$ by a factor of up to 40-fold greater than β5, and was initially developed as an anti-cancer agent but later moved into the autoimmune arena. While the effects ofONX0914 are not being evaluated in hematologic malignancies, it is interesting to note that this IPSI is in clinical trials for systemic lupus erythematosus[27], inflammatory bowel disease[28], and rheumatoid arthritis[29]. ONX0914 has an immunosuppressive effect in that it inhibits IL-1, IL-6, and TNF production.[27–29] ONX0914 treatment also blocked the production of interferon-γ and IL-2 by T-cells, and IL-23 by activated monocytes. It remains to be seen whether targeting the immunoproteasome will be an effective strategy in autoimmune disorders, but the data from the pre-clinical studies with murine models are encouraging.

Other IPSIs

Ho et al. rationally designed and synthesized a number of small molecular inhibitors to target the $\beta1_i$ immunoproteasome subunit.[36] These compounds, which were analogs of dihydroeponemycin, were shown to irreversibly inhibit $\beta1_i$ activity with a very high specificity in models of angiogenesis and prostate cancer. Specific binding to $\beta1_i$ led to inhibition of the ChT-L activity assigned to the $\beta5_i$ subunit, which is felt to be key for the potency of a proteasome inhibitor. Additionally, the specificities of their compounds were further confirmed when they showed that they preferentially inhibited growth of prostate cancer cells that over-expressed the $\beta1_i$ subunit when compared to a $\beta1_i$-null cell line.[36] These data further indicate that cells overexpressing immunoproteasome catalytic subunits are good selective targets for IPSIs in both solid and hematologic malignancies.

Conclusions

The immunoproteasome remains a compelling target for cancer therapy, but the available data are somewhat contradictory. Parlati et al.[14] found a lack of cell death in the hematologic cell lines they tested when using only CPSIs or IPSIs alone, and only when dual inhibition was achieved, such as with the non-specific agent carfilzomib, did cell death occur. In contrast, Kuhn et al.[32], Singh et al.[38], and Ho et al.[39] showed that IPSIs were by themselves sufficiently potent to induce programmed cell death both *in vitro* and *in vivo*. This discrepancy may in part be due to the variability between model systems in the extent to which cells express the immunoproteasome, since those models that express higher $20S_i$ levels seem most sensitive to IPSIs. Also, differences in the subunit specificities for the various inhibitors tested may be responsible as well, since direct inhibitors of $\beta5_i$ did not induce apoptosis, while those that target $\beta1_i$, which may suppress other sites as well through allosteric interactions, could be more potent. Finally, attention needs to be paid to the experimental conditions used, since high concentrations of an IPSI could result in loss of specificity with resultant inhibition of the constitutive proteasome as well, mimicking the effects of non-specific inhibitors. Further studies will therefore be needed to more fully

determine the potential of the immunoproteasome as a therapeutic target in cancer, as well as in autoimmune-related diseases.

Acknowledgments

D.J.K. would like to acknowledge support from the National Cancer Institute (1K99 CA149140). R.Z.O. would like to acknowledge support from the Leukemia & Lymphoma Society (6096-07), the Multiple Myeloma Research Foundation, and the National Cancer Institute (P50 CA142509).

Abbreviations

CHOP	C/EBP homologous protein
CPSI	constitutive proteasome-specific inhibitor
IL	interleukin
IPSI	immunoproteasome-specific inhibitors
PARP	poly(ADP)-ribose polymerase
shuIL6R	soluble human IL-6 receptor
TNF	tumor necrosis factor
UPP	ubiquitin proteasome pathway

References

1. Buchdunger E, Zimmermann J, Mett H, et al. Inhibition of the Abl protein-tyrosine kinase in vitro and in vivo by a 2-phenylaminopyrimidine derivative. Cancer Res. 1996; 56(1):100–104. [PubMed: 8548747]
2. Deininger MWN, Druker BJ. Specific targeted therapy of chronic myelogenous leukemia with imatinib. Pharmacol Rev. 2003; 55(3):401–423. [PubMed: 12869662]
3. Richardson PG, Barlogie B, Berenson B, et al. A phase 2 study of bortezomib in relapsed, refractory myeloma. N Engl J Med. 2003; 348(26):2609–2617. [PubMed: 12826635]
4. Richardson PG, Sonneveld P, Schuster MW, et al. Bortezomib or high-dose dexamethasone for relapsed multiple myeloma. N Engl J Med. 2005; 352(24):2487–2498. [PubMed: 15958804]
5. O'Connor OA, Wright J, Moskowitz C, et al. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol. 2005; 23(4):676–684. [PubMed: 15613699]
6. Bianchi G, Oliva L, Cascio P, et al. The proteasome load versus capacity balance determines apoptotic sensitivity of multiple myeloma cells to proteasome inhibition. Blood. 2003; 113(13): 3040–3049. [PubMed: 19164601]
7. Voorhees PM, Dees EC, O'Neil B, Orlowski RZ. The proteasome as a target for cancer therapy. Clin Cancer Res. 2003; 9(17):6316–6325. [PubMed: 14695130]
8. Lowe J, Stock D, Jap B, Zwickl P, Baumeister W, Huber R. Crystal structure of the 20S proteasome from the archaeon T. acidophilum at 3.4 A resolution. Science. 1995; 268(5210):533–539. [PubMed: 7725097]
9. Unno M, Mizushima T, Morimoto Y, et al. Structure determination of the constitutive 20S proteasome from bovine liver at 2.75 A resolution. J Biochem. 2002; 131(2):171–173. [PubMed: 11820928]
10. Rivett AJ. The multicatalytic proteinase. Multiple proteolytic activities. J Biol Chem. 1989; 264(21):12215–12219. [PubMed: 2745438]
11. Kisselev AF, Akopian TN, Castillo V, Goldberg AL. Proteasome active sites allosterically regulate each other, suggesting a cyclical bite-chew mechanism for protein breakdown. Mol Cell. 1999; 4(3):395–402. [PubMed: 10518220]

12. Jiang H, Monaco JJ. Sequence and expression of mouse proteasome activator PA28 and the related autoantigen Ki. Immunogenetics. 1997; 46(2):93–98. [PubMed: 9162094]
13. Noda C, Tanahashi N, Shimbara N, Hendil KB, Tanaka K. Tissue distribution of constitutive proteasomes, immunoproteasomes, and PA28 in rats. Biochem Biophys Res Commun. 2000; 277(2):348–354. [PubMed: 11032729]
14. Parlati F, Lee SJ, Aujay M, et al. Carfilzomib can induce tumor cell death through selective inhibition of the chymotrypsin-like activity of the proteasome. Blood. 2009; 114(16):3439–3447. [PubMed: 19671918]
15. Gaczynska M, Rock KL, Spies T, Goldberg AL. Peptidase activities of proteasomes are differentially regulated by the major histocompatibility complex-encoded genes for LMP2 and LMP7. Proc Natl Acad Sci USA. 1994; 91(20):9213–9217. [PubMed: 7937744]
16. Gaczynska M, Rock KL, Goldberg AL. Gamma-interferon and expression of MHC genes regulate peptide hydrolysis by proteasomes. Nature. 1993; 365(6443):264–267. [PubMed: 8396732]
17. Rock KL, Goldberg AL. Degradation of cell proteins and the generation of MHC class I-presented peptides. Annu Rev Immunol. 1999; 17:739–779. [PubMed: 10358773]
18. Heink S, Ludwig D, Kloetzel P-M, Krüger E. IFN-γ-induced immune adaptation of the proteasome system is an accelerated and transient response. Proc Natl Acad Sci USA. 2005; 102(26):9241–9246. [PubMed: 15944226]
19. Shin EC, Seifert U, Kato T, et al. Virus-induced type I IFN stimulates generation of immunoproteasomes at the site of infection. J Clin Invest. 2006; 116(11):3006–3014. [PubMed: 17039255]
20. Hallermalm K, Seki K, Wei C, et al. Tumor necrosis factor-alpha induces coordinated changes in major histocompatibility class I presentation pathway, resulting in increased stability of class I complexes at the cell surface. Blood. 2001; 98(4):1108–1115. [PubMed: 11493458]
21. Boes B, Hengel H, Ruppert T, Multhaup G, Koszinowski UH, Kloetzel PM. Interferon gamma stimulation modulates the proteolytic activity and cleavage site preference of 20S mouse proteasomes. J Exp Med. 1994; 179(3):901–909. [PubMed: 8113682]
22. Strehl B, Seifert U, Kruger E, Heink S, Kuckelkorn U, Kloetzel PM. Interferon-gamma, the functional plasticity of the ubiquitin-proteasome system, and MHC class I antigen processing. Immunol Rev. 2005; 207:19–30. [PubMed: 16181324]
23. Rivett AJ, Hearn AR. Proteasome function in antigen presentation: immunoproteasome complexes, peptide production, and interactions with viral proteins. Curr Protein Pept Sci. 2004; 5(3):153–161. [PubMed: 15180520]
24. Chu-Ping M, Vu JH, Proske RJ, Slaughter CA, DeMartino GN. Identification, purification, and characterization of a high molecular weight, ATP-dependent activator (PA700) of the 20 S proteasome. J Biol Chem. 1994; 269(5):3539–3547. [PubMed: 8106396]
25. Hoffman L, Pratt G, Rechsteiner M. Multiple forms of the 20 S multicatalytic and the 26 S ubiquitin/ATP-dependent proteases from rabbit reticulocyte lysate. J Biol Chem. 1992; 267(31): 22362–22368. [PubMed: 1331052]
26. Song X, von Kampen J, Slaughter CA, DeMartino GN. Relative functions of the alpha and beta subunits of the proteasome activator, PA28. J Biol Chem. 1997; 272(44):27994–28000. [PubMed: 9346951]
27. Ichikawa HT, Conley T, Muchamuel T, et al. Novel proteasome inhibitors have a beneficial effect in murine lupus via the dual inhibition of type 1 interferon and autoantibody secreting cells. Arthritis Rheum. 2012; 64(2):493–503. [PubMed: 21905015]
28. Basler M, Dajee M, Moll C, Groettrup M, Kirk CJ. Prevention of experimental colitis by a selective inhibitor of the immunoproteasome. J Immunol. 2010; 185(1):634–641. [PubMed: 20525886]
29. Muchamuel T, Basler M, Aujay MA, et al. A selective inhibitor of the immunoproteasome subunit LMP7 blocks cytokine production and attenuates progression of experimental arthritis. Nat Med. 2009; 15(7):781–787. [PubMed: 19525961]
30. Visekruna A, Joeris T, Seidel D, et al. Proteasome-mediated degradation of IκBα and processing of p105 in Crohn disease and ulcerative colitis. J Clin Invest. 2006; 116(12):3195–3203. [PubMed: 17124531]

31. Arastu-Kapur S, Anderl JL, Kraus M, et al. Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res. 2011; 17(9):2734–2743. [PubMed: 21364033]
32. Kuhn DJ, Hunsucker SA, Chen Q, Voorhees PM, Orlowski M, Orlowski RZ. Targeted inhibition of the immunoproteasome is a potent strategy against models of multiple myeloma that overcomes resistance to conventional drugs and nonspecific proteasome inhibitors. Blood. 2009; 113(19): 4667–4676. [PubMed: 19050304]
33. Orlowski M, Cardozo C, Eleuteri AM, Kohanski R, Kam CM, Powers JC. Reactions of [C-14]-3,4-dichloroisocoumarin with subunits of pituitary and spleen multicatalytic proteinase complexes (proteasomes). Biochemistry. 1997; 36(45):13946–13953. [PubMed: 9374874]
34. Cardozo C, Michaud C, Orlowski M. Components of the bovine pituitary multicatalytic proteinase complex (proteasome) cleaving bonds after hydrophobic residues. Biochemistry. 1999; 38(30): 9768–9777. [PubMed: 10423257]
35. Kuhn E, Addona T, Keshishian H, et al. Developing multiplexed assays for troponin I and interleukin-33 in plasma by peptide immunoaffinity enrichment and targeted mass spectrometry. Clin Chem. 2009; 55(6):1108–1117. [PubMed: 19372185]
36. Ho YK, Bargagna-Mohan P, Wehenkel M, Mohan R, Kim KB. LMP2-specific inhibitors: chemical genetic tools for proteasome biology. Chem Biol. 2007; 14(4):419–430. [PubMed: 17462577]
37. Kisselev AF, Garcia-Calvo M, Overkleeft HS, et al. The caspase-like sites of proteasomes, their substrate specificity, new inhibitors and substrates, and allosteric interactions with the trypsin-like sites. J Biol Chem. 2003; 278(38):35869–35877. [PubMed: 12815064]
38. Singh AV, Bandi M, Aujay MA, et al. PR-924, a selective inhibitor of the immunoproteasome subunit LMP-7, blocks multiple myeloma cell growth both in vitro and in vivo. Br J Haematol. 2011; 152(2):155–163. [PubMed: 21114484]