Comment on Roccaro et al, page 4051

Aiming at WM with both barrels blocked

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Proteasome inhibition has proven to be a successful therapeutic strategy in B-cell malignancies including WM. In this issue of Blood, Roccaro and colleagues report preclinical studies, providing the rationale for clinical investigation of a novel orally available proteasome inhibitor in this disease.1

How can a therapy be targeted to cancer if the target is in every normal cell? Because proteasomes are found in all nucleated as well as anucleated cells, it could be argued that this target is even less cancer-specific than that of conventional DNA-damaging chemotherapy. However, proteasome inhibitors clearly have made an impact in the treatment of many types of cancer and none bigger than in B-cell malignancies. Recent studies of proteasome inhibition including those presented here suggest that the answer may be that the target is unique in B-cell malignancies including Waldenstrom macroglobulinemia (WM). These cells have 2 types of proteasomes that must be inhibited to induce apoptosis, and now this can be achieved with an orally available agent.

WM is an IgM-secreting lymphoplasmacytic lymphoma with bone marrow involvement that accounts for 1% to 2% of hematologic malignancies.2 Because WM shares many of the biological characteristics of both lymphomas and multiple myeloma, WM patients have benefited from therapeutic advances in both diseases. Current therapeutic approaches in WM include the use of rituximab-containing regimens as well as bortezomib, which had single-agent activity and recently was shown to be highly active in combination with rituximab and dexamethasone.3 However, bortezomib had to be discontinued in 61% of the patients on this study because of peripheral neuropathy.3 Peripheral neuropathy associated with bortezomib use has led to the development of several second-generation proteasome inhibitors, including the irreversible inhibitor, carfilzomib, that did not induce dose-limiting neuropathy in a phase 1 study of 29 patients with hematologic malignancies.4 Now, an analog of carfilzomib has been developed that is orally bioavailable5 and appears to hold promise for the treatment of WM.

In the current study, ONX0912 (formerly PR-047) induced growth inhibition and apoptosis in a dose-dependent fashion in both WM and low-grade IgM-secreting lymphoma cell lines as well in cells from 4 WM patients while having no effect on B cells from healthy donors.1 Using a unique ELISA-based activity assay to capture proteasome-active sites,6 the investigators demonstrated that both the constitutive proteasome and the immunoproteasome are expressed in WM cells at significantly higher levels than normal B cells. While the catabolic requirements of normal resting B cells and IgM-secreting cells are likely to be different, the data do support the notion that...
the target may be more relevant based on the biology of an IgM-secreting cell. Indeed, previous studies using the same assay did not demonstrate a difference in proteasome levels in CD138+ cells from normal donors and myeloma patients. More importantly, the data demonstrate inhibition of both proteasomes. Because the immunoproteasome is only expressed constitutively in hematopoietic cells, this may provide an explanation as to why B-cell malignancies are exquisitely sensitive to this class of therapeutics.

The constitutive proteasome and immunoproteasome are nearly identical, with both possessing 3 catalytic subunits in their 20S barrel. These subunits have chymotryptic-like, trypsin-like, and caspase-like activity, with the chymotryptic-like subunit being the target of ONX0912 as well as the primary site of action of bortezomib. However, the subunits that contain these activities are different in the constitutive proteasome (ß5, ß2, and ß1, respectively) and the immunoproteasome (LMP7, MECL1, and LMP2, respectively). These changes result in differences in the roles that each proteasome plays. In addition to degradation of the unneeded, damaged, or misfolded proteins from the cytoplasm and endoplasmic reticulum, the constitutive proteasome regulates signal transduction, cell-cycle progression, and apoptosis. While initially it was believed that the reason for the different subunits in the immunoproteasome was to optimize peptide generation during antigen processing for MHC class I presentation, recent evidence suggests that additional roles exist for the immunoproteasome including cytokine production. Amazingly, the use of proteasome-specific inhibitors demonstrates that the cellular processes controlled by each proteasome are in part distinct as selective inhibition of the constitutive proteasome does not affect IL-23 production in monocytes. However, these selective inhibitors do not induce apoptosis when used individually, thus overlapping functions are likely to exist as well. Therefore, normal and transformed B cells may be dependent on both proteasomes and explain why these cells are so sensitive to proteasome inhibition. It will be important to determine which processes are uniquely controlled versus those that are regulated by both proteasomes to further understand the biology of diseases like WM (see figure).

These findings may also explain why it is hard to alter the effect of proteasome inhibition by blocking the individual effects of proteasome inhibition. For example, the authors demonstrate activation of the unfolded protein response yet pharmacologic inhibition of this response provides minimal, albeit statistically significant protection from ONX0912-induced death. This is similar to previous studies that demonstrated that myeloma cells are significantly more sensitive to bortezomib than NF-kB inhibition. In contrast, JNK activation is downstream of both proteasomes and may provide clues to how proteasome inhibition results in cell death. More intriguing may be the effects of ONX0912 on the bone marrow stromal cells that can support WM cell growth and survival through the production of cytokines. ONX0912 does not kill these cells but inhibits cytokine production. It remains to be determined which proteasomes are present in these cells. However, it adds to the promise of this class of agents for the treatment of B-cell malignancies such as WM, and now this double-barreled inhibition can be achieved with an orally available drug.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES


Proteomics unravels platelet function

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In this issue of Blood, Schulz and colleagues report promising findings using differential proteomics as a discovery tool to identify functionally important proteins in platelet activation. The authors identify several proteins that change in abundance upon platelet activation. These findings implicate several novel pathomechanisms relevant to platelet activation and identify novel potential therapeutic targets for platelet inhibition. Platelets have a pivotal role in thrombosis, vascular repair, and inflammatory reactions. They lack nuclei and genomic DNA and are thus not amenable to most of the classical cell, molecular biology, and genomic techniques. For the identification of proteins and novel protein functions, proteomic studies are therefore the method of choice. Proteomic approaches are further favored by easy access to large amounts of platelet proteins via blood donations. Platelets possess a pre-mRNA splicing machinery and also have remnants of megakaryocyte-derived mRNA, which both can result in rapid translation upon platelet activation. Thus, proteomic comparisons between resting and activating platelets are expected to reveal differences in abundance of proteins. Technical advances in proteomic technologies and recent achievements in generating protein data repositories hold great promise for important discoveries in platelet research.

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