something that was previously assumed but now is substantiated by data and statistical analyses.

So how can we take advantage of this knowledge? Since specific genetic targets are not defined in CLL, Mcl-1 seems to be an appropriate biomolecule to therapeutically manipulate. As shown in the figure, Mcl-1 protein production and maintenance are dependent on several pathways. At the apical level, the microenvironment provides factors that dramatically increase this protein in CLL cells. Hence, a strategy that interferes with interaction of microenvironment and CLL cells is a logical approach. Production of Mcl-1 through these signals is carried via increased transcription of the MCL-1 gene. Transcription and polyadenylation inhibition, albeit not selective, is an approach that works because of AU-rich elements in the transcript of Mcl-1, which leads to its rapid turnover. The N-terminal region of Mcl-1 protein contains 2 PEST domains that are rich in proline, glutamic acid, serine, and threonine residues, resulting in a short half-life of the protein and making translation inhibition and rapid degradation of endogenous Mcl-1 via proteasome pathway a viable option to reduce the protein level. Mcl-1 is also essential during early lymphoid development and is abundantly expressed in the germinal center B-cell compartment. Pin kinase and Akt-PI3-kinase pathways and downstream of BlyS have been identified to maintain the Mcl-1 levels in B cells. The roles of these pathways and consequence of their perturbations need to be investigated in malignant lymphocytes. Similarly, work is needed on posttranslational modification leading to increased or decreased half-life of Mcl-1 protein. Finally, and probably most intriguingly, small molecule antagonists of Mcl-1 protein that bind to the BH3 domain releasing proapoptotic proteins provide a new avenue of research and therapeutics.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Comment on Liu et al, page 3835

Why is CLL refractory to bortezomib?

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In this issue of Blood, Liu and colleagues present evidence that flavonoids present in plasma may compromise the ability of the proteasome inhibitor bortezomib to induce apoptosis of CLL cells. Bortezomib is a dipeptide boronate proteasome inhibitor which induces apoptosis of chronic lymphocytic leukemia (CLL) cells in vitro. However, treatment of CLL patients with this agent generated no objective responses. In this issue of Blood, Liu et al present evidence indicating that the discrepancy between the in vitro and in vivo observations may be accounted for by a chemical reaction between bortezomib and plasma components. Initial observations showed that while CLL cells cultured in media containing 10% fetal calf serum were induced to apoptosis by bortezomib, this action was dramatically compromised in the presence of 50% fresh human plasma. Further studies showed that the dietary flavonoid quercetin, which is present in plasma, blocked CLL cell killing by bortezomib. This action was confirmed by carrying out multiple cellular and molecular assays which showed that bortezomib-induced CLL cell killing occurs via the classic mitochondria-dependent intrinsic apoptotic pathway and that these mechanisms are compromised by quercetin.

The blocking effect of flavonoids is attributable to a direct chemical reaction between the boronate moiety of bortezomib and adjacent hydroxyl groups present on the B ring of some, but not all, flavonoids. First, quercetin and myricetin, which contain adjacent hydroxyls, effectively blocked bortezomib’s cytotoxic action, while kaempferol and apigenin, which do not contain adjacent hydroxyls, failed to do so. Second, cell killing by the proteasome inhibitors MG-132 and lactacystin, proteasome inhibitors which do not possess a boronate group, was unaffected by flavonoids, which compromised apoptosis induction by the boronate compounds, bortezomib and MG-262. Finally, data obtained using Raman spectroscopy were consistent with a direct chemical reaction between quercetin and bortezomib.

Liu et al also show that boric acid reacts with quercetin and abolishes its inhibitory action on bortezomib-induced apoptosis. Boric acid also abrogated the protective action of plasma, suggesting that the ability of plasma to compromise CLL cell killing by bortezomib is indeed attributable to flavonoids. However, questions remain concerning the actual levels of flavonoid species present in plasma. While plasma levels of quercetin are insufficient on their own to account for the quenching effect, other flavonoid species are present in plasma. It would therefore be of interest to identify and quantify those species which are reactive with bortezomib. The concentration issue is particularly relevant since the actions of quercetin are significantly dose-dependent: While 20 μM quercetin effectively blocked killing of CLL cells by bortezomib, concentrations of 40 μM or greater actually induced apoptosis, an action that may be explained by the apparently paradoxical observation that quercetin itself may, like bortezomib, inhibit the β5 subunit of the proteasome.

The studies here raise a further question: If plasma flavonoids effectively neutralize the cytotoxic actions of bortezomib on CLL cells, how is the observed effectiveness of this agent in multiple myeloma explained? The authors suggest that interactions between flavonoids and CLL cells on the one hand and myeloma...
cells on the other may be different. Specifically, they propose that quercetin may augment the sensitivity of multiple myeloma cells to bortezomib, so as to compensate for chemical inactivation of the proteasome inhibitor by the flavonoid. It would be of interest to explore this possibility via comparative mechanistic studies on CLL and myeloma cells.

The authors suggest several strategies which may allow further development of proteasome inhibitors for the treatment of CLL. First, decreasing plasma flavonoid concentrations by dietary manipulation may, if achievable, be of value in enhancing in vivo toxicity of bortezomib. Second, the observation that boric acid can compete with the reaction between quercetin and bortezomib raises the possibility that the blocking effect of flavonoids may be neutralized prior to bortezomib treatment. A third option would be to explore the possible therapeutic use of proteasome inhibitors which lack a boronate moiety.

In some respects, the data of Liu et al echo earlier observations that plasma blocked apoptosis induction of CLL cells following treatment with chlorambucil. However, the mechanism of blockade in this earlier study was different than that described here and involved inhibition of intracellular apoptotic-signaling pathways by albumin following induction of DNA damage, rather than by direct inactivation of chlorambucil by reaction with plasma components. Nevertheless, these studies emphasize the sometimes profound effects of human plasma on cytotoxic mechanisms and open the way for the design of strategies which may result in more effective therapeutic use of existing cytotoxic agents.

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