Targeting Plasma Cells with Proteasome Inhibitors: Principles from Primates

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Tarlinton and Hodgkin1 first proposed plasma cell (PC)–targeted therapies for autoimmune disease in 2004, but basic research studies did not emerge until 2008.2 The first PC-targeted therapy in human transplantation was published in 2008 using a proteasome inhibitor (PI)–based regimen in refractory antibody-mediated renal allograft rejection (AMR).3 These initial human PC-targeted therapies used bortezomib, a first generation reversible PI approved for the PC malignancy multiple myeloma. Over the past decade, the pharmaceutical industry has committed significant investments in PC-based therapies in multiple myeloma that have transformed outcomes, whereas investments and progress in transplantation have been much less significant.

Increasing clinical experience with PI-based PC-targeting strategies in transplantation has shown variable results between disease states and patient populations (early versus late AMR, AMR versus desensitization, and adults versus children). PI therapy in murine models of autoimmune disease has shown similar variabilities and also shown that PC populations vary between disease models and thus, may represent another source of variability.2,4–7 Transplant experiences with bortezomib in humans have indicated that HLA antibodies resulting from distant HLA exposures (i.e., HLA antibodies produced by long-lived plasma cells [LLPCs]) are more refractory to PI therapy compared with HLA antibodies resulting from primary exposure (i.e., HLA antibodies produced by plasmablasts [PBs]).8 Therefore, clinical conditions primarily dependent on bone marrow niche–resident LLPCs, such as chronic HLA sensitization, have become a focus for progressive targeting of resistant PC populations in specific conditions (for example, in desensitization).9 However, human LLPC resistance and immunologic compensatory mechanisms in response to PI therapy for non-oncologic indications remain elusive. This knowledge gap hampers our current ability to further proceed with biologic and pathway-based approaches to further enhance responses to PI therapies in transplantation.

In this issue of the Journal of the American Society of Nephrology, Kwun et al.10 present important new evidence, providing novel mechanistic insights into immunologic responses after PI therapy in a chronically MHC-sensitized primate model. Their primate model accurately represents chronically HLA-sensitized humans and provides a validated model for clinical “desensitization,” enabling study of PI therapy for reducing HLA antibodies.11 In this study, bortezomib therapy significantly depleted bone marrow plasma cells (BMPCs); however, antidonor antibodies were not reduced in serum. Mechanistic studies showed impressive proliferation in germinal center (GC) B cells and T follicular helper (Tfh) cells after PI therapy. IL-21 and IL-6 levels were not increased; however, significant increases in BAFF levels were observed.

Deletion of antibody-secreting BMPCs by bortezomib without a reduction in DSA levels was intriguing. Given the relatively long $t_{1/2}$ of Ig in primates (3–4 weeks), bortezomib therapy would have had to reduce Ig production by a significant degree over a substantial period of time for a DSA reduction to have been observed. Because a DSA reduction was not observed, the question as to how antibody levels were maintained must be considered. Several potential explanations exist to explain the observation. One potential explanation involves the compensatory GC-proliferative response, which would have been expected to generate new DSA-secreting PBs that could be observed transiently in peripheral blood or bone marrow. Although the authors did not study the generation of new PBs in association with the GC expansion, such a response could have potentially restored DSA production lost when PI therapy depleted BMPCs.

Other possibilities that could explain the DSA stability despite PC depletion include (1) de novo generation of PCs from GC memory B cells, (2) generation of new PCs from proliferation within a bone marrow precursor PC population, and (3) retrotadifferentiation of terminally differentiated bone marrow niche–resident LLPC population and reacquisition of proliferative capacity. Demonstration of transient appearance of new PBs in the GC and/or peripheral blood and/or spleen is an important approach that should be used in future studies to help determine the mechanism(s) responsible for recovery of DSA production after BMPC depletion. Consistent with the possibility that new DSA-producing
PCs may derive from bone marrow rather than GC, preliminary evidence from single-cell RNA seq studies of CD138+ BMPCs in humans indicates that proliferating BMPC populations are increased during carfilzomib desensitization therapy.12

PIs induce GC disruption via their ability to induce cell cycle arrest in proliferating cell populations. Possibilities must, therefore, be considered that would explain the paradoxical observation of GC expansion. First, the authors note that bortezomib dosing was adequate to induce PC depletion and that higher dosing may not have been possible due to toxicity. Second, bortezomib is a reversible inhibitor, and therefore, at the time of postbortezomib testing, the macaques may have had several days without effective bortezomib levels, such that post-treatment sampling was indeed measuring a reflexive response.

Desensitization experiences in humans have confirmed a concept long known to immunologists and vaccinologists (viz., that interventions designed to reduce serum antibody levels are routinely accompanied by antibody rebound). This observation suggests that homeostatic mechanisms exist, whereby the immune system keeps antibody levels at relatively constant levels over long periods of time. The primary mechanisms by which this occurs remain incompletely defined, and the authors suggest that PC depletion removes PC suppression of Tfh activity13 or alternatively, via BAFF-mediated effects on B cells.

Kwun et al.10 propose that combination therapies be considered for enhancement of PI therapy (e.g., by blocking of the B cell/Tfh proliferative responses with drugs that block BAFF, GC initiation, or GC compensation). Given the complexity that derives from PI therapy for chronic humoral responses, it would be surprising that single-agent therapy would be sufficient. On the basis of this study, costimulatory blockade by agents, such as belatacept, would provide targeted approaches for future combinatorial therapies for enhancing PI therapy.

The cellular and biochemical mechanisms by which PIs perturb immunologic states are complex. PIs exert effects on multiple cell types beyond PCs, including immature and mature B cells, T cell populations, and dendritic cells. At the cellular level, PIs potently induce unfolded protein response (UPR) genes, which in turn, may mediate autophagy or if unrelied, a terminal UPR response with caspase-dependent apoptosis. PIs also induce cell cycle arrest in proliferating cells and inhibit NF-κb–mediated IL-6 and TNF production, thereby diminishing critical growth factors in B cell development at multiple levels.

In addition, PIs also exert pleiotropic effects on PCs, thereby creating an additional level of complexity. PIs clearly deplete PCs, but they also induce marked derangement in endoplasmic reticulum morphology,5 suggesting that they also likely inhibit Ig production directly or even indirectly via UPR induction. During an active immune response, it is also likely that PIs induce apoptosis in rapidly proliferating PBs and memory B cell populations and therefore, are capable of inducing GC disruption.

Finally, PIs themselves vary considerably in their biochemical properties with respect to (1) reversibility of proteolytic activities; (2) differential effects on chymotryptic, tryptic, and caspase-like enzymatic activity; and (3) differential effects on constitutive versus immunoproteasome enzymatic activity. Therefore, it will be important to test other PIs in future primate studies. Clearly, additional studies are needed to help place the various effects of PIs into perspective.

Opportunities exist for enhancing PC therapies solely at the level of the proteasome, including use of irreversible PIs (as opposed to the reversible PI bortezomib). Early experiences suggest that the second generation irreversible PI carfilzomib may have enhanced efficacy and reduced toxicity.12 Another second generation PI, ixazomib, may also provide advantages over bortezomib. To date, PI therapy has focused primarily on inhibition of proteolytic activity—a distal event in proteasome-based protein degradation. More recently, there is a new focus on more proximal events (viz., inhibition of ubiquitin binding [by the RPN 13 inhibitor14] and inhibition of ubiquitin degradation by deubiquitinase inhibitors [known as DUBs]). Obviously, blocks in series may lead to potential for synergy.

Beyond the proteasome, enhancement of ER stress and modulation of ER stress–associated pathways may provide alternative approaches for enhancing PI therapy. Proximal UPR inhibitors are under development, particularly IRE-1 inhibitors. Similarly, autophagy inhibition by one of a number of approaches may eliminate a major potential pathway for bypassing the proteasome, which may limit efficacy.

Accumulation of almost a decade of experience with clinical PI therapy for antihumoral responses in humans is now being complemented by human mechanistic studies and murine and primate studies. This study in primates indicates that second generation PI-based regimens will require additional components designed to prevent PC reconstitution. Therefore, in a manner analogous to development of combination regimens for T cell–based immunosuppression in transplantation, antihumoral therapies will likely be combination therapies consisting of induction (i.e., PC depletion) and maintenance (i.e., prevention of PC repletion) phases. Finally, experience in multiple myeloma has shown that development of rationally designed, targeted combination therapies with PIs can lead to major progress in treating a challenging disease.

Primate studies provide an extremely valuable resource for generation of potentially clinically translatable observations. This study provides incisive and important new information that will not only aid in understanding these complex humoral immune responses but also, likely lead to new approaches for enhancing innovative PC-targeting regimens for human disease. Future results from this intriguing primate model are eagerly awaited.
ILC2: There’s a New Cell in Town

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A new type of innate cell that resembles a lymphocyte but lacks a T cell receptor has been identified and is called an innate lymphoid cell (ILC). These cells are found predominantly in epithelial tissues, such as the gut, the lung, and the skin, and have important roles in immunity, infection, and homeostasis.1 ILCs are divided into functional groups ILC1, ILC2, and ILC3 that match cytokines, transcription factors, and surface receptors in classes that have parallels with T helper (Th) subsets Th1, Th2, and Th17.2 ILCs have much in common with Th2 cells and a major role in protection from disease and possibly, driving autoimmune disease. Given the role of ILCs in other organs, they are highly likely to have an important role in kidney disease.

In this issue of the Journal of the American Society of Nephrology, Riedel et al.3 explore the role of ILC2s in human and mouse kidney as well as disease models. ILCs have recently been recognized as one of the key immune defenses against infection.4,5 The ILC2 subset is thought to play an important role in the pathogenesis of asthma,6 hyper-reactive lung7, and intestinal8 and skin9 diseases through the production of IL-5 and IL-13. IL-33, one of the IL-1 family of cytokines, induces rapid expansion of ILC2 cells. A key feature of mouse and human ILC2s is the expression of the IL-33 receptor ST2. In addition to its various cytokines, ILC2s, like regulatory T cells (Tregs), secrete amphiregulin, which acts to protect tissues.8 However, the action of ILCs in nonbarrier organs, such as kidney, is still largely unknown.

Riedel et al.3 show that IL-33 receptor-positive ILC2s form a major subtype of ILCs in both human and murine kidneys. They show that ILCs constitute 0.5%–3% of leukocytes of both human and murine kidney, with IL-33 receptor-positive ILC2s constituting the major subtype. Short-term IL-33 treatment led to sustained expansion of the kidney-residing ILC2s. The expansion seems to be occurring within tissue-resident ILCs rather than in peripheral lymphoid organs. IL-33–induced expansion of renal ILC2 numbers was shown to ameliorate glomerulosclerosis in an adriamycin mouse model of CKD.10 This finding is

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REFERENCE


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