Proteasome inhibitor-based therapy for antibody-mediated rejection

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The development of donor-specific anti-human leukocyte antigen antibodies (DSAs) following renal transplantation significantly reduces long-term renal graft function and survival. The traditional therapies for antibody-mediated rejection (AMR) have provided inconsistent results and transient effects that may be due to a failure to deplete mature antibody-producing plasma cells. Proteasome inhibition (PI) is a novel AMR therapy that deletes plasma cells. Initial reports of PI-based AMR treatment in refractory rejection demonstrated the ability of bortezomib to deplete plasma cells producing DSA, reduce DSA levels, provide histological improvement or resolution, and improve renal allograft function. These results have subsequently been confirmed in a multicenter collaborative study. PI has also been shown to provide effective primary AMR therapy in case reports. Recent studies have demonstrated that PI therapy results in differential responses in early and late post-transplant AMR. Additional randomized studies are evaluating the role of PI in transplant induction, acute AMR, and chronic rejection in renal transplantation. An important theoretical advantage of PI-based regimens is derived from several potential strategies for achievement of synergy.

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The development of anti-human leukocyte antigen (HLA) antibodies (Abs) specific for the renal allograft is associated with diminished allograft survival, regardless of whether the donor-specific anti-HLA antibody (DSA) is *de novo* or anamnestic in origin.[1–3] However, prompt and complete DSA elimination may improve allograft survival.[4,5]

There are currently no immunosuppressive agents approved by the Food and Drug Administration for antibody-mediated rejection (AMR) treatment. Historically, AMR has been treated with a variety of approaches, including intravenous immunoglobulin (IVIg), therapeutic plasma exchange, rabbit anti-thymocyte globulin, and rituximab. These approaches, however, do not deplete the source of Ab production—the mature plasma cell.[6] This limitation may contribute to the suboptimal and unreliable results observed with non-plasma cell-depleting agents.

Given the need for reliable and durable elimination of anti-HLA alloantibodies, considerable efforts are being focused on developing new antihumoral therapies. Recent reports have described the use of the proteasome inhibitor bortezomib (Millennium Pharmaceuticals, Cambridge, MA) in treating AMR. The utility of bortezomib was first demonstrated in the treatment of refractory AMR,[7] and has subsequently been shown to provide effective primary therapy for AMR in case reports.[5] The purpose of this review is to discuss the role of proteasome inhibition (PI) in AMR treatment.

PROTEASOMES AND PI

The 26S proteasome is a large multimeric enzymatic structure present in the cytosol of all eukaryotic cells. The proteasome structurally resembles a cylinder and consists of a 20S core with 19S regulatory subunits capping each end (Figure 1). The 20S core consists of four stacked heptameric rings, with two beta rings surrounded by two alpha rings. Residing inside of the cylinder, the proteolytic activity of the proteasome is protected from the cytosol. Three distinct proteolytic activities exist within the beta ring: chymotryptic-like, tryptic-like, and postglutamyl (that is, caspase-like) hydrolyzing activity.[8,9] Each alpha ring provides a restricted opening, which limits the entry of proteins destined for proteolysis.

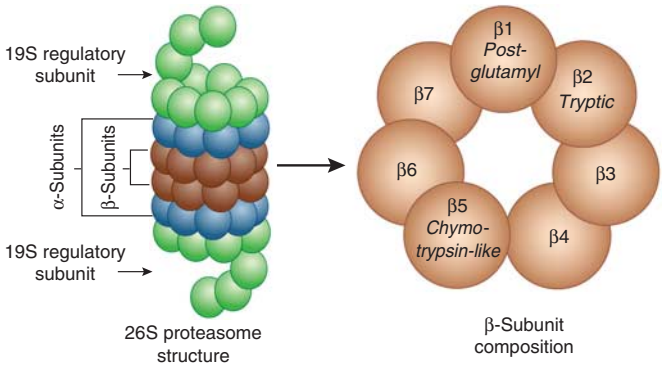

Figure 1 | 26S Proteasome structure.

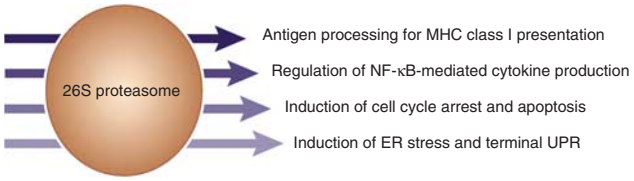

Figure 2 | Primary mechanisms of proteasome inhibitor-mediated immunomodulation. ER, endoplasmic reticulum; MHC, major histocompatibility complex; NF-κB, nuclear factor-kappa B; UPR, unfolded protein response.

Proteasomal function provides cellular homeostasis via the selective degradation of misfolded proteins, cell-cycle regulatory proteins, transcription factors, and inhibitory molecules.[8,9] Bortezomib interrupts this homeostasis through reversible binding of the β5-subunit, the site of chymotryptic-like proteolytic activity, within the 20S core of the 26S proteasome.[9] PI results in dysregulation of numerous cellular processes, including antigen processing and mitosis regulation (Figure 2).

The ubiquitin–proteasome system is the major intracellular mechanism for production of peptide fragments of suitable length for presentation on major histocompatibility complex (MHC) class I molecules.[10,11] This process is facilitated by the 26S proteasome; however, in inflammatory states, differing β-subunits with differing enzymatic activities are produced that provide an alternative array of peptides for antigen presentation. Cytokine stimulation via interferon-γ enhances immunoproteasome activity as well as upregulates cell surface MHC class I expression. Through PI, the degree of antigen processing and presentation on MHC class I molecules can be inhibited.[10,11]

There are four major physiological effects of proteasome inhibitor therapy that are thought to be primarily responsible for its immunomodulatory effects and likely its effectiveness in AMR. These include: (1) inhibition of nuclear factor-kappa B (NF-κB) activity, (2) inhibition of proliferation and induction of apoptosis via cell cycle arrest, (3) induction of apoptosis via ER stress, and (4) inhibition of Class I MHC expression via reduction in endogenous peptide production.

Cell cycle progression is mediated through a precisely choreographed series of enzymatic events via cyclins. Throughout mitosis, the synthesis and subsequent degradation of cyclins is required for cell cycle progression at multiple checkpoints. The ubiquitin–proteasome system maintains appropriate levels of these key cyclins.[12–14] Interruption of this precise series of events (for example, viaPI) results in cell cycle arrest and apoptosis.

Protein synthesis and folding are managed within the endoplasmic reticulum (ER) and are vital for the maintenance of normal cellular homeostasis. When a critical threshold of unfolded or misfolded proteins accumulates within the ER, a number of protective responses are generated that comprise the unfolded protein response (UPR). The UPR is characterized by three major processes: (1) reduction in synthesis of nascent proteins, (2) upregulation of ER chaperones and foldases, and (3) expression of proteins that constitute the ER-associated degradation pathway and facilitate protein trafficking from the ER to the proteasome.[15,16] PI potently induces an UPR due to the accumulation of high levels of misfolded proteins that have been targeted for proteasomal digestion. If the accumulation of misfolded proteins is not controlled, a terminal UPR results, which consists of mitochondrially and caspase-mediated cellular apoptosis.[15]

PI also effectively inhibits production of pro-inflammatory cytokines. NF-κB-mediated transcription of pro-inflammatory cytokines is regulated through the binding of inhibitor nuclear factor-kappa B (IκB) to NF-κB. IκB is downregulated by ubiquitination and subsequent proteasomal degradation. Therefore, PI results in IκB stability and a resulting diminishment in production of pro-inflammatory cytokines such as interleukin (IL)-2, IL-6, IL-10, IL-13, interferon-γ, and tumor necrosis factor-α.[13,17] Reduction in the pro-inflammatory cytokines by PI has also been demonstrated in anti-CD3-stimulated T cells.[18] Reduction in the inflammatory cytokine milieu improves overall inflammation and allograft damage, ultimately strengthening the role of PI in immunologically mediated damage post transplantation.

PI IN ANIMAL MODELS OF HUMORALLY MEDIATED AUTOIMMUNE DISEASE

Proteasome inhibitors have been evaluated in animal models of humorally mediated autoimmune disease. These studies have provided an enhanced understanding of the effects of systemic proteasome inhibitor therapy. A disease that closely mimics systemic lupus erythematosus develops spontaneously in NZB/W F1 mice.[19] Autoantibodies to double-stranded DNA mediate glomerulonephritis and play a critical role in disease progression in this model. Ultimately, these double-stranded DNA autoantibodies and the associated glomerulonephritis result in significant mortality in this model. Neubert and colleagues utilized this animal model to assess the response to PI with bortezomib and elucidate the mechanism by which disease amelioration is induced.

Flow cytometry analysis of CD138+ short- and long-lived plasma cells from spleen and bone marrow demonstrated a bortezomib-mediated decrease in both cell populations, with an overall 95% reduction in the bone marrow plasma cell population.[19] Importantly, when compared to treatment with dexamethasone or cyclophosphamide, bortezomib demonstrated a greater reduction in the total number of splenic- and bone marrow-derived plasma cells. However, only bortezomib significantly reduced serum levels of anti-double-stranded DNA immunoglobulin G levels, which may have resulted from the induction of a terminal UPR in plasma cells, as evidenced by the induction of the protein-folding chaperone BiP. Clinically, reduction in plasma cells and anti-double-stranded DNA Abs result in amelioration of glomerulonephritis and prolonged survival. Pathological examination of bortezomib-treated animals demonstrated an absence of glomerulitis, vasculitis, and Ab deposition that was present in control animals. Proteinuria, a marker of renal dysfunction, also significantly improved with bortezomib treatment. Notably, infectious complications were not apparent as mice treated with bortezomib survived for more than 10 months without signs of infection. Given the evidence presented, bortezomib appears to possess considerable potential as a plasma cell–depleting agent.

Vanderlugt *et al.*[20] evaluated the effects of PI in a murine model (SJL/J mouse) of relapsing experimental autoimmune encephalomyelitis (an experimental model of multiple sclerosis induced through immunization with myelin protein eptitopes). Mice treated with the proteasome inhibitor PS-519 showed improvement in paralysis scores, incidence of clinical relapse decreased, delay type hypersensitivity reaction, and spinal cord histology.

Palombella *et al.*[21] studied the effects of PI in a Lewis rat polyarthritis model induced by intraperitoneal injection of group A Streptococcal cell wall peptidoglycan and polysaccharide. This polyarthritis model is characterized by NF-κB-mediated upregulation of cell adhesion molecules and proinflammatory cytokines with histology that closely mimics rheumatoid arthritis. Utilizing several methodologies to assess disease severity, Palombella *et al.*[21] demonstrated improvement in clinical manifestations of polyarthritis (assessed by the total arthritis index) in bortezomib-treated animals. Additionally, average hind paw volume, an objective measure of polyarthritis, was also markedly reduced in bortezomib-treated animals. Finally, histology of hind paw joints at necropsy demonstrated a reduction in cellular infiltrates as well as an attenuation of the degradation of articular cartilage and erosion of subchondral bone.

BORTEZOMIB THERAPY IN TRANSPLANTATION

Three studies have examined potential roles for proteasomes and PIs in transplant rejection models. Luo *et al.*[18] evaluated the PI dipeptide boronic acid (DPBA) and found it to suppress T-cell proliferation and IL-2, IL-6, IL-10, IL-13, and γ-interferon production *in vitro* in response to anti-CD3 monoclonal Ab. Short-term administration of DPBA (16 days) was found to prolong murine heart allograft survival for up to 35 days compared with 7 days in control mice. This group of investigators also examined DPBA in a murine islet allograft model.[22] In this experience, the authors first demonstrated that DPBA suppressed mixed lymphocyte reactions and cytotoxic T-cell generation *in vitro*. In murine islet transplant recipients, a 17-day DPBA course provided 50% islet allograft survival at 60 days, whereas control mice demonstrated islet allograft rejection at 7 days. No effect of DPBA was found on islet function following glucose challenge. In a more recent study, administration of bortezomib on day 20 following MHC-mismatched heart transplantation prolonged cardiac allograft survival to 31.7 days compared with 6.3 days in untreated controls.[23] The authors also found lower levels of anti-MHC class I and II Abs at 7 days following transplantation. In a chronic AMR rat cardiac transplant model, administration of bortezomib beginning at 60 or 80 days following transplantation reduced anti-donor MHC class I and II Abs. Histological improvements were also observed with bortezomib administration, including reduction in C4d expression, interstitial fibrosis, and vasculopathy.[23]

Recent clinical experiences have provided evidence for the ability of proteasome inhibitor-based regimens to reverse AMR. In the first report of bortezomib in renal transplantation, we treated a series of patients with refractory mixed acute rejection, defined as biopsy-proven rejection meeting the Banff criteria for both AMR and acute cellular rejection.[7] In this report, six patients treated with refractory mixed acute rejection were treated for eight rejection episodes. Each rejection episode was treated with one cycle of bortezomib (4 doses of 1.3 mg/m^2). Two patients received an additional bortezomib cycle for recurrent rejection. In all cases, these mixed acute rejection episodes had previously failed multiple therapies, including plasmapheresis, rituximab, rabbit anti-thymocyte globulin, and IVIg.

Immunodominant DSA (iDSA), which is defined as the highest-level DSA at the time of rejection diagnosis, was used as the marker of AMR treatment efficacy. Bortezomib therapy reduced iDSA levels by more than 50% in all cases.[7] This includes one case where bortezomib was administered alone with plasmapheresis and resulted in >70% reduction in the iDSA and a >90% reduction in other DSA specificities. Interestingly, the majority of iDSA specificities were MHC class II specificities, and half of all the rejection episodes had an HLA-DQ specificity for the iDSA. Previously, it has been suggested that MHC class II Abs are generally more refractory to treatment.[24]

Renal function improved or remained stable in the majority of rejection episodes. Also notably, renal allograft biopsies showed that all patients experience resolution or improvement in acute cellular rejection, including one patient with a Banff grade IIA acute cellular rejection, in which a severe endotheliitis completely resolved with bortezomib treatment.[7]

In this initial experience, the toxicity profile of bortezomib was reasonable, as only two patients were reported to experience adverse events.[7] One patient experienced transient thrombocytopenia and diarrhea (which resolved with anti-diarrheal therapy). Another patient experienced febrile neutropenia (without infection), which resulted in holding of the final dose of bortezomib. Neutropenia was attributed to the additive effects of multiple immunosuppressants and anti-infective agents in this patient. Opportunistic infection and malignancy were not reported for any patients. This case series demonstrates the ability of bortezomib to provide a marked reduction in DSA levels, resolution of allograft biopsy histology, and improvement in allograft function.

A subsequent report described the use of bortezomib in combination with daily plasmapheresis and IVIg in two positive cross-match kidney recipients with AMR.[25] One patient was highly sensitized pre-transplant and exhibited a flow cross-match channel shift >300. This patient received a perioperative plasmapheresis and IVIg protocol, with AMR developing during treatment with this protocol. The second patient was less sensitized initially, and therefore did not receive the perioperative plasmapheresis and IVIg protocol. Both patients received bone marrow biopsies at the time of AMR and 1 week following treatment to assess therapeutic response to AMR treatment with bortezomib.

Both patients had numerous alloantibody specificities at the time of AMR, both donor specific and third-party anti-HLA Abs.[25] Ab levels were measured, by single antigen anti-HLA beads, at the time of rejection and 1 year following bortezomib treatment. In both patients, the total number of allospecificities and levels of remaining Abs were reduced in response to treatment with bortezomib. Bone marrow aspirates in these two patients showed that the percentage of plasma cells in bone marrow declined in response to bortezomib therapy. One year post transplant, both patients maintain normal renal function and transplant glomerulopathy was absent on protocol biopsies. Notably, total immunoglobulin levels were normal in both patients despite the absence of DSA-producing plasma cells.

Bone marrow biopsies done during AMR treatment in these patients provide a unique perspective on the effects of bortezomib on bone marrow–resident plasma cells.[25] An ELISpot assay for the detection of antitetanus Ab demonstrated a greater than 50% reduction in Ab production in bone marrow–derived plasma cells treated with bortezomib.

More recently, PI with bortezomib has been evaluated as the primary therapy for AMR in a series of two patients.[5] Both patients were treated with the combination therapy, which consisted of bortezomib, rituximab, and plasmapheresis. Bortezomib ($1.3\,mg/m^2$) was given on treatment days 1, 4, 8, and 11. Patients received plasmapheresis before each bortezomib dose and every other day for 3 sessions beginning 72 h after the final bortezomib dose. Rituximab ($375\,mg/m^2$) was administered on treatment day 1 following bortezomib and plasmapheresis. A representation of the histological and immunological improvement seen with bortezomib-based therapy is depicted in Figure 3.

The first patient developed AMR on post-transplant day 13 following his third kidney transplant.[5] Although DSAs were absent before the transplant, an acute rise in creatinine level on post-transplant day 13 prompted a renal allograft biopsy, which demonstrated C4d immunostaining of the peritubular and glomerular capillaries (Figure 3a and b). High levels of DSA were detected on single HLA-antigen bead testing by Luminex. Bortezomib-based treatment was initiated on post-transplant day 14. Following treatment, DSA was undetectable (Figure 3) and repeat renal allograft biopsy on post-transplant day 28 demonstrated a reduction in glomerular and peritubular capillary C4d staining (Figure 3c and d). Serum creatinine levels returned to pre-rejection baseline and proteinuria was not observed.

The second patient to receive bortezomib-based primary therapy for AMR was a 41-year-old woman who received a one-haplotype-matched living donor kidney transplant from her son.[5] DSA was absent before transplant, but a low-level DSA was detected through surveillance on post-transplant day 7. DSA continued to rise, and on post-transplant day 13 a renal allograft biopsy demonstrated faint peritubular capillary and strong glomerular capillary C4d staining. DSA was also markedly increased, and treatment was initiated on post-transplant day 15. Plasmapheresis was done before the first two bortezomib doses, but otherwise held to reduce risk of bleeding complications from recent surgical procedures. Treatment response included DSA elimination, return of serum creatinine levels to baseline, and resolution of proteinuria. Approximately 2 months following initial treatment, the patient developed recurrent DSA elevation without renal dysfunction, which was treated with a second course of bortezomib-based therapy. Again, DSA was decreased below level of detection and serum creatinine level remained stable. Interestingly, the iDSA of the first rejection episode (anti-HLA B7) was absent during the second rejection episode. This suggests that plasma cell clones responsible for producing anti-HLA B7 Abs were durably depleted by the first bortezomib course.

This first report on bortezomib primary therapy also demonstrated the relative safety of bortezomib-based AMR treatment protocols.[5] Mild anemia was reported for both patients, which likely represented an artifact of end-stage renal disease given the early post-transplant course. Additionally, the second patient experienced mild nausea, vomiting, and diarrhea. During the second rejection episode, the second patient also experienced transient peripheral neuropathy, which completely resolved within 3 days. In short, these cases illustrate the ability of bortezomib-based therapy for early post-transplant AMR to completely eliminate DSA, improve renal biopsy histology, and return renal function to baseline, with limited toxicity.

Recently, Legendre and colleagues[26] presented clinical data suggesting that bortezomib alone is not effective in reducing DSA levels in a case series of four patients. Conclusions from

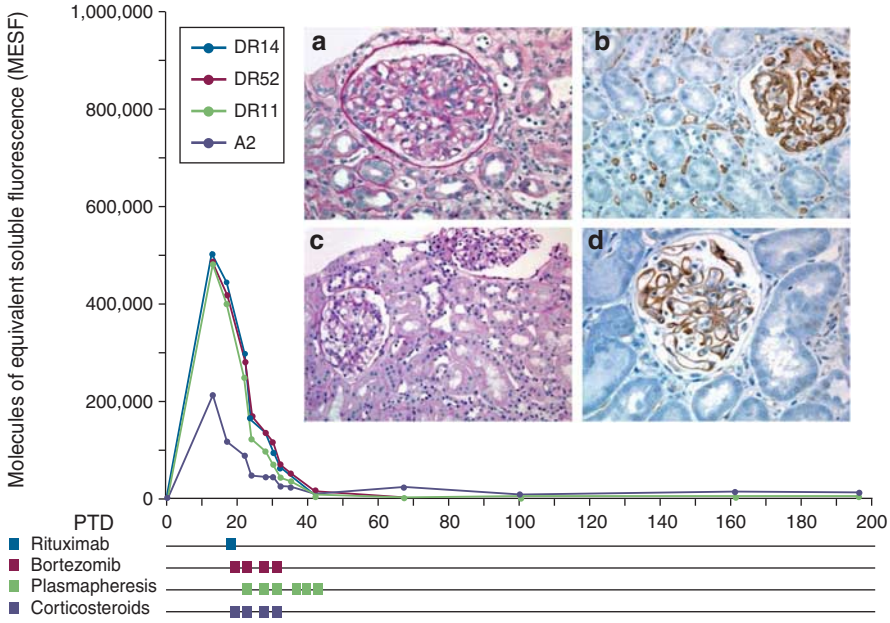

Figure 3 | Response of donor-specific anti-human leukocyte antigen (HLA) antibody (DSA) levels to treatment with bortezomib (expressed as molecules of equivalent soluble fluorescence (MESF)). The blue line represents immunodominant DSA (HLA DR14) and other colored lines represent non-immunodominant DSA. Renal allograft biopsy pre-treatment (PTD 13) demonstrates (**a**) mild acute tubular injury, mild glomerulitis, and mild inflammation of peritubular capillaries with no evidence of acute cellular rejection (ACR), and (**b**) immunostain for C4d shows strong diffuse peritubular and glomerular capillary deposition. Post-treatment (PTD 28) renal allograft biopsy in patient 1 demonstrates (**c**) no evidence of ACR, and (**d**) immunostain for C4d shows rare, faint peritubular capillary labeling and strong glomerular capillary deposition. Original magnification: (**a**, **c**) $\times$200; (**b**, **d**) $\times$400. PTD, post transplant day.

this study, however, are significantly compromised by several considerations. First, patients were treated with bortezomib for clinically silent AMR (also termed subacute or subclinical AMR), a lesion that has only recently been described.[27–29] As such, subclinical AMR has not yet been recognized in the Banff grading system as a distinct entity, and its responsiveness to any AMR therapy remains to be defined. More importantly, no renal allograft biopsy was performed in these four patients earlier than 1 month prior to bortezomib therapy (actual times include biopsy at 1, 1, 3, and 5 months before bortezomib therapy). Therefore, it is impossible to ascertain what lesion was actually being treated in these patients. Moreover, no patient received a follow-up biopsy, thereby precluding assessment of the histological response to therapy. In addition, since serum creatinine level was not elevated in any patient, renal function could not be evaluated as an efficacy criterion. Therefore, the only evaluable criterion to assess efficacy in this study were DSA levels. We have previously shown that DSA reductions in response to any type of therapy for late post-transplant AMR are diminished.[30]

The authors assert that there are many possibilities for the apparent lack of effect on DSA levels. One factor may be that corticosteroids (which were eliminated by the authors from the bortezomib regimen) may be synergistic with bortezomib.[26] Given that oncologists almost universally use corticosteroids in conjunction with bortezomib, it is surprising that the removal of corticosteroids from this treatment protocol was attempted. Also, the relatively stable and low level of DSA indicates a low metabolic state of plasma cells, which may offer protection from PI-induced UPR. Plasma cells, which are metabolically active and produce high levels of immunoglobulin, have been shown to be more susceptible to UPR induction by PI.[31] Additionally, late post-transplant AMR may represent a more difficult clinicopathological etiology to treat as it is likely predominated by bone marrow survival niche–resident plasma cells.[5]

Of interest, patients treated in this study exhibit a unique side-effect profile.[26] Three out of the four patients treated experienced bilateral conjunctivitis. Patients also complained of a generalized weakness that lasted up to a month following the final dose of bortezomib. More typically, nausea, vomiting, and diarrhea were each experienced by one patient. Hematological toxicities and infectious complications were not noted in these patients.

More recently, large experiences with proteasome inhibitor–based treatment of AMR have been published.[30,32] We compared the results of bortezomib-based therapy by examining the results in 13 early and 17 late ($>$6 months) post-transplant AMR episodes. This comparison has illustrated the dichotomous nature of AMR responses.[30] Patients with early post-transplant AMR were more likely to have been sensitized before transplant, and to demonstrate a larger reduction in DSA levels and greater improvement in renal allograft biopsy histology with bortezomib treatment. In all early AMR patients, bortezomib-based therapy resulted in

significant improvement in renal function. Interestingly, late AMR was more often associated with a DSA directed against the HLA-DQ specificity, and pre-treatment DSA levels were significantly higher than in patients with early AMR. However, late AMR episodes demonstrated a lesser magnitude of improvement in DSA levels and renal function. These findings indicate that late post-transplant AMR tends to be less responsive than early acute AMR to treatment.

Flechner *et al.*[32] have reported their experience with bortezomib-based AMR treatment in kidney transplant recipients. In this experience, patients with higher serum creatinine levels at the time of initiation of AMR therapy demonstrated reduced therapeutic responses, suggesting that more severe or more established AMR episodes may demonstrate diminished therapeutic responses.

Initial results from a multicenter collaborative utilizing a single proteasome inhibitor-based regimen were very similar to those obtained in our initial report on the effectiveness of bortezomib in AMR.[33] Eighty-one patients treated for 96 AMR episodes with a common bortezomib-based regimen demonstrated substantial DSA reductions with bortezomib-based therapy, with more than half of the patients achieving a $>50\%$ reduction in iDSA level. In addition, this collaborative experience demonstrated that the bortezomib-based regimen reversed AMR in adult kidney, kidney/pancreas, and pediatric heart transplant recipients. It is important to note that in bortezomib-based regimens plasmapheresis has been performed every third day immediately before bortezomib therapy. This is in contrast to IVIg-based regimens where plasmapheresis has traditionally been reported to be performed more frequently—either daily or on alternate days.

Bortezomib has also been shown to reduce DSA and third party anti-HLA Ab levels in patients without AMR.[34] In this report, 11 patients transplanted under a clonal stimulation and deletion protocol were treated for anti-HLA Abs appearing within the first 100 days post-transplant. Treatment included bortezomib ($1.3\,mg/m^2$) on days 1, 4, 8, and 11 of treatment. Methylprednisolone 250 mg was administered concomitantly with each bortezomib dose. In 6 of the 11 cases, rituximab was included as adjuvant therapy. In the majority of patients treated, anti-HLA Ab levels, including those directed against the donor, were reduced below 1000 mean fluorescence intensity in a median time of 24 days from treatment initiation.[34] Two patients, both with peak Ab levels $>10{,}000$ mean fluorescence intensity, did not reduce Ab titers to <1000 mean fluorescence intensity. However, following treatment, both patients exhibited a $>50\%$ reduction in primary Ab. Notably, at the last follow-up, Ab levels in both refractory cases had rebounded to near peak levels. However, despite high levels of Ab, serum creatinine level remains stable throughout in both cases. However, given the relatively short length of follow-up, it will be prudent to examine the long-term sequelae of elevated DSA levels in these patients. Significant data supporting the detrimental effects of DSA on allograft survival exist.[1–3]

Bortezomib therapy was tolerated well in this patient population.[34] The most prevalent adverse event was diarrhea; however, the authors indicate that the etiology of this cannot be distinguished owing to the endemic nature of diarrhea in the region. Thrombocytopenia was reported in all patients treated with bortezomib. Additionally, one patient reported generalized weakness, which resolved following treatment. Opportunistic infections were not observed in this patient population.

PI IN TRANSPLANTATION: CONSIDERATIONS FOR ONGOING AND FUTURE STUDIES

As with all immunosuppressive agents developed to date, the efficacy of bortezomib may be enhanced when used within the context of combination regimens. Rituximab is an agent that has considerable potential for enhancing the efficacy of proteasome inhibitor-based regimens. Rituximab alone has been used previously to treat AMR and offers the ability to deplete naive and memory B-cell precursors of plasma cells. However, rituximab-based regimens have not demonstrated the ability to durably reduce DSA levels,[35] possibly because of its lack of effect on plasma cells.[6,25]

Therapeutic plasma exchange also has been used in the treatment of rejection, but acts simply to mechanically remove preformed Abs in circulation. The combination of all three aforementioned agents has demonstrated significant efficacy.[5] It is suggested that in this combination therapeutic plasma exchange serves two roles: (1) reduction in negative feedback inhibition experienced by alloantibody-producing plasma cells, thus making them more metabolically active and susceptible to targeting by PI, and (2) providing a timely and accurate reflection of the true Ab production capability by removing existing circulating Abs.[5]

Our approach toward examining the efficacy of bortezomib for AMR treatment has been conservative and we have restricted the initial patient population to a maximum of two cycles of therapy.[5,7] However, in the multiple myeloma population a median of six cycles of bortezomib have been used with a reasonable adverse event profile, and many patients are treated for a year or more.[36] As greater experience with bortezomib in the transplant population is gained, maximization of therapy will be evaluated. Additional cycles of bortezomib may result in a greater durability in alloantibody depletion and improved long-term outcomes. Recent data indicated that up to four cycles are well tolerated in highly sensitized waitlist patients undergoing bortezomib-based desensitization.[37]

Bortezomib is a first-in-class proteasome inhibitor developed for its anti-neoplastic properties. Currently, there are several second-generation proteasome inhibitors under development for use in the oncology population.[38] Desired improvements include a greater activity against the proteasome, as well as an improved safety profile. Ease of administration is also one improvement being targeted, with the orally bioavailable proteasome inhibitor PR-047 currently being investigated.[38]

Bortezomib may be combined with other agents to enhance therapeutic efficacy. As with a number of cancer agents, drug resistance may be mediated by *p*-glycoprotein; therefore *p*-glycoprotein inhibitors may be useful in enhancing bortezomib efficacy. Similarly, autophagy inhibitors, such as histone deacetylase inhibitors, may also be useful in potentiating bortezomib therapy. Other new agents that inhibit humoral responses may be reasonable to use in combination with bortezomib, such as BAFF or April inhibitors or IL-6 inhibitors.

The role of PI in transplantation will not be limited to AMR treatment. It will also be important to ensure that the development of treatment strategies be done under carefully designed and executed clinical trials. Currently, we are conducting controlled trials examining the use of bortezomib in desensitization protocols, induction strategies, and chronic rejection (http://www.clinicaltrials.gov). Additionally, the use of bortezomib is being evaluated in patients receiving solid organ transplants other than kidney.[39–41]

We recently outlined a number of issues related to clinical trial design and conduct for antihumoral agents seeking the FDA approval for acute and chronic AMR therapy, desensitization, and AMR prevention.[42] For AMR in particular, development of end points will be important. To date, we have used three criteria for assessing therapeutic responses when treating AMR: (1) renal function, (2) renal allograft histology, and (3) DSA levels. Currently, the assay for assessing DSA levels is being optimized and validated by several groups. Our preliminary data indicate that serum creatinine levels and repeat allograft biopsy could likely be reasonable end points; however, a careful examination of Banff components will be necessary.

CONCLUSION

The elimination of DSA improves renal allograft outcomes, but the question of which approach is most suited for the abrogation of DSA remains to be established. Historical therapies, such as plasmapheresis, rituximab, rabbit antithymocyte globulin, and plasmapheresis, do not deplete anti-HLA Ab-secreting plasma cells.[6,7] Proteasome inhibitor-based regimens have provided an alternative strategy for AMR treatment. An important advantage of proteasome inhibitor-based strategies for targeting plasma cells is the large number of potential synergistic strategies that can be employed to enhance their effects on plasma cells.

The greatest advantage of proteasome inhibitor-based protocols over IVIg-based regimens is the number of strategic approaches that exist for achieving synergy with proteasome inhibitors. A fundamental understanding of the biology of protein degradation pathways and clinical development of new agents targeting differing components of these pathways are requisite steps in developing synergistic plasma cell depletional combination regimens. Therein lies a very promising pathway for addressing the previously almost impenetrable barrier presented by the production of extraordinarily high levels of HLA alloantibodies by bone marrow niche–resident plasma cell clonal populations. The future of antihumoral therapies in clinical transplantation has never been more exciting.

DISCLOSURE

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