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REVIEW

Proteasome Inhibition and Allogeneic Hematopoietic Stem Cell Transplantation: A Review

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The proteasome and its associated ubiquitin protein modification system have proved to be an important therapeutic target in the treatment of multiple myeloma and other cancers. In addition to direct antitumor effects, proteasome inhibition also exerts strong effects on nonneoplastic immune cells. This indicates that proteasome inhibition, through the use of agents like bortezomib, could be used therapeutically to modulate immune responses. In this review we explore the emerging data, both preclinical and clinical, highlighting the importance of proteasome targeting of immunologic responses, primarily in the context of allogeneic hematopoietic stem cell transplantation (HSCT), both for the control of transplant-related toxicities like acute and chronic graft-versus-host disease (aGVHD, cGHVHD), and for improved malignant disease control after allogeneic HSCT.

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INTRODUCTION

Over the last decade, the rationale for allogeneic hematopoietic stem cell transplantation (HSCT) has evolved from primarily a means to rescue patients after myeloablative (MA) high-dose conditioning chemo/radiotherapy with an immune-hematopoietic graft from a disease-free donor, to a means of providing adoptive cellular immunotherapy to induce curative graft-versus tumor (GVT) responses. The number of allogeneic transplants performed annually continues to rise, in part because of the increasing frequency of preparative regimens using reduced-intensity conditioning (RIC) in older/sicker patients. However, despite its ability to provide meaningful long-term disease-free and overall survival (DFS, OS) for patients, allogeneic HSCT remains a procedure with considerable treatment-related morbidity and mortality (TRM), and malignant disease relapse is not uncommon. Graft-versus-host disease (GVHD) remains the most frequent complication of allogeneic HSCT, with clinically significant (grade II-IV) acute GVHD (aGVHD) occurring in ~35% of matched related donor (MUD) transplants, and up to 50% of unrelated or alternative donor transplants, whereas chronic GVHD (cGVHD) can affect up to 60% of recipients who survive beyond 100 days after matched donor allogeneic HSCT [1].

aGVHD was originally defined as disease appearing within the first 100 days posttransplant, with cGVHD being more delayed. It is now clear that they can overlap temporally after transplant, especially since the introduction of RIC regimens [2]. aGVHD and cGVHD are now categorized by their clinical presentations and not by the time of onset [3]. aGVHD typically targets the skin, intestine, and liver (the lung can also be targeted), whereas cGVHD has more protean manifestations, which can target skin and mucosa, lung, liver, hematopoietic, musculoskeletal/serous tissues, and exocrine glands. To a degree, it resembles collagen vascular diseases [4] and has some autoimmune characteristics including autoantibody formation.

Despite differences in clinical presentation and management, aGVHD and cGVHD are primarily believed to arise from donor alloreactive T cell responses, which also underlie curative GVT responses. The pathophysiology of cGVHD is less understood than aGVHD. In part, this is because of the lack of good animal models that represent cGVHD's full pathologic spectrum. Recently, however, a new model has been described that approximates the clinical manifestations of human cGVHD [5]. Indeed, limitations in preclinical studies may account, in part, for

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the finding that improvements in allogeneic HSCT preparative regimens and prophylaxis of aGVHD have not had a significant impact on the incidence of cGVHD [1]. Front-line treatment for both aGVHD and cGVHD consists of steroid administration, despite its limited efficacy and significant cumulative toxicity. Novel strategies are needed to better control GVHD without having a significant impact on the associated GVT response.

Pathophysiology of GVHD and GVT

Mouse models have been instrumental in understanding the role of cytokines and the T cell subsets in aGVHD and GVT responses. These models have demonstrated how the immune subsets develop postallogeneic HSCT and produce mediators that play a critical part in these 2 processes. Both donor $CD4^+$ and $CD8^+$ effector T cells can utilize perforin to mediate lethal GVHD [6]. The perforin and TRAIL cytotoxic pathways, but not tumor necrosis factor (TNF)-α, are associated with $CD8^+$ T cell-mediated GVT [7-9]. It should be noted, however, that murine models of GVT are predominately $CD8^+$ T cell mediated, which may skew the interpretation of findings. In appropriate mouse major histocompability complex (MHC) II^+ tumor models, $CD4^+$ T cells can also mediate GVT [10-12].

Historically, aGVHD has been considered a primarily T helper 1 (Th1)/T cell 1 (Tc1) type process, based on the predominance of cytotoxic T cell-mediated pathology and increased production of Th1-type cytokines including interferon (IFN)-γ, whereas cytokines that polarize donor T cells to Th2 (e.g., granulocyte-colony stimulating factor [G-CSF], interleukin [IL]-4, IL-18) can reduce aGVHD [13-16]. However, this may be an oversimplification, because the developmental blockade of Th1 or Th2 phenotypes through the use of cells lacking critical transcription factors [17], or ablation of either IL-2 producing (Th1-type) or IL-4 producing (Th2-type) donor T cells following the onset of clinical symptoms of GVHD [18], demonstrated that both Th1 and Th2 type donor T cells can induce aGVHD. In addition, in aGVHD, the production of the Th1/Tc1 type cytokine, IFN-γ, by both $CD4^+$ and $CD8^+$ donor T cells limits the severity of the disease in recipient mice after MA conditioning [19-21]. However, the cytokine is needed for the retention of GVT activity in a murine leukemia model [19]. The pro-inflammatory cytokine TNF-α has been shown to be an effector of both aGVHD and cGVHD based on the ability of TNF blockers to ameliorate disease in clinical trials [22,23]. In murine models, the absence or blockade of TNF-α in $CD4^+$ T cell-mediated murine aGVHD can ameliorate disease and result in a reduction in GVT to a greater degree than in $CD8^+$ T cell-mediated disease [9,24] even though both $CD4^+$ and $CD8^+$ T cells can produce the cytokine. The rationale for this observation is unclear. Recently, a third T cell subset, Th17, defined by IL-17 production and antagonized by IFN-γ, has been recognized. However, the role of IL-17 and/or Th17 cells in aGVHD has been controversial [25-27]. In contrast, cGVHD has been considered by some a Th2/Tc2-type disease based on its autoimmune-like features, the presence of autoantibodies [28], and the predominance of Th2-type cytokines in mouse models [29]. Recently, the contribution of Th17 cells to cGVHD has also been demonstrated [25]. Other cellular mediators include the $CD4^+CD25^+Foxp3^+$ T regulatory cell subset, which has been shown to suppress both aGVHD and cGVHD [30-33]; and B cells, which have been implicated in cGVHD. The contribution of B cells to the pathophysiology of the disease has gained significant interest with the observation that rituximab therapy resulted in durable improvement in a proportion of patients with refractory cGVHD [34-36]. More recently, prophylactic use of rituximab has shown promise [37]. In the context of B cell dysfunction, elevated levels of B cell activating factor (BAFF) of the TNF family has been seen to correlate strongly with development cGVHD [38,39]. BAFF is a cytokine that promotes survival and activation of B cells. BAFF levels have been shown to be elevated immediately following autologous and allogeneic HSCT [38,39], but wane with B cell recovery and reduction in other biomarkers of inflammation in patients that do not go on to develop cGVHD [38]. Our knowledge of the pathobiology of aGVHD and cGVHD continues to develop.

Proteasome Inhibition in Cancer

The proteasome is a large protein complex containing an adenosine 5′-triphosphate-dependent protease that plays a critical function in the degradation of ubiquitinated proteins [40-43]. It also plays a key regulatory function in many vital cellular processes by degrading proteins involved in cell cycle [44-46], responses to oxidative stress, major histocompatibility comples (MHC) class I- restricted antigen processing [47], and regulation of gene expression (including NF-κB through the stabilization of ubiquinated IkB [48]). Inhibition of the proteasome can result in arrest of cell cycling and can trigger intrinsic apoptotic pathways [48-52]. Bortezomib (Velcade®, formerly PS-341) is a dipeptidyl boronic acid that binds to, and blocks the activity of the catalytic site of the 26S proteasome [53]. Bortezomib was the first proteasome inhibitor to enter clinical trials, and was subsequently granted approval for use in treatment of patients with multiple myeloma (MM) [42,54,55]. It is now also approved for the treatment of relapsed mantle cell lymphoma [56]. Novel proteasome inhibitors such

as NPI-0052 and carfilzomib are currently in clinical trials [57-63].

The first recognition that bortezomib had antitumor activity was based on direct cytotoxic effects on the tumor cell. Bortezomib, and proteasome inhibition in general, can block the degradation of a number of cell cycle-regulated proteins. Consequently, treatment of cells with bortezomib results in the accumulation of cells in the G2-M phase of the cell cycle [64-66]. Arrested cells are eventually killed by apoptotic pathways as demonstrated by caspase activation and DNA fragmentation [65,66]. In addition to blocking the degradation of cell cycle regulatory proteins, blockade of NF-κB activation, through stabilization of its inhibitor, IκB, is another important target for proteasome inhibition-induced cell death. Stimulation of quiescent cells through a number of various stimuli including cytokines, viruses, antigens, or oxidants leads ultimately to ubiquitation and subsequent protolytic degradation of IκB [48,52,67]. Loss of cytoplasmic IκB results in translocation of NF-κB to the nucleus where it promotes transcription of a number of target genes affecting the inflammatory response, including many cytokines and cell adhesion molecules [52]. NF-κB is also essential for cell viability in a number of cell types through the induction of gene transcription for inhibitors of apoptosis [68-73]. Thus, failure to remove IκB through proteasome-mediated clearance results in the sustained inhibition of NF-κB-mediated transcription in bortezomib-treated cells.

In addition to direct tumor cytotoxicity, bortezomib can sensitize tumor cells to chemotherapy-induced apoptosis through a variety of mechanisms. Bortezomib has been shown to enhance cellular cytotoxicity with histone deacetylase (HDAC) inhibitors through a reactive oxygen species (ROS)-dependent mechanism [74,75] with cisplatin through upregulation of pro-apoptotic proteins [76], with topoisomerase-I inhibitors in an NF-κB-independent mechanism [77], and with doxorubicin and melphalan (Mel) by lowering the apoptotic threshold to these agents [78]. Bortzomib has also been reported to sensitize cells to DNA-damaging agents [79]. In addition, proteasome inhibitors have been shown, in some instances, to overcome chemoresistance, such as cisplatin via induction of endoplasmic reticulum stress [80] and adhesion-mediated drug resistance to agents such as doxorubicin [78], vincristine, and dexamethasone [81].

Proteasome Inhibition and Immune Responses

In addition to direct cytotoxic effects on tumor cells following bortezomib treatment, bortezomib has been shown to sensitize target cells to immune-mediated killing through TRAIL/DR5 [73,82-84] and Fas/FasL pathways [85,86] on natural killer (NK) and $CD8^+$ T effector cells. Although there are some discrepancies between studies that are most likely because of differential responses of various tumor cell lines to bortezomib and/or sensitivity to killing pathways, in general, they demonstrate that bortezomib can upregulate expression of the death receptors Fas and DR5 on tumor cells. This expression of death receptors, which in combination with the increased sensitivity to apoptosis, because of downregulation of antiapoptotic molecules can promote cytotoxic T lymphocyte (CTL) and NK killing of bortezomib-treated tumor cells. In addition, bortezomib treatment can lead to downregulation of cell surface expression of MHC I as a result of decreased antigen processing [47,82]. This treatment can promote targeting of tumor cells to NK cell killing. Finally, tumor cell death via direct cytotoxic action of bortezomib may increase the immunogenicity of the dying cells through the expression of heat-shock protein 90 (Hsp90) on the cell surface [87], which may result in further expansion of the tumor-specific immune response.

It is important to note that, although bortezomib treatment can sensitize tumors to cytotoxic lymphocytes, it also can suppress immune function by a variety of mechanisms. Induction of pro-inflammatory cytokines and chemokines are an essential component for establishing a productive immune response. Gene regulation of many of these proteins is dependent on NF-κB translocation to the nucleus. Blockade of NF-κB activity through proteasome inhibition can result in the inability of immature dendritic cells (DCs) to mature into activated immunostimulatory DCs [88-90], whereas activated DCs may be less susceptible to the immunomodulatory effects of bortezomib [89]. In addition, differentiating monocyte-derived DCs may be vulnerable to bortezomib-induced apoptosis [91,92].

Sensitivity of lymphohematopoietic tumors to bortezomib cytotoxity, in some instances, correlates to sensitivity of the normal cellular counterpart. Thus, cellular levels of immunoglobulin biosynthesis have been shown to correlate with sensitivity of both MM cells [93] and plasma cells [94] to bortezomib cytotoxicity. This sensitivity of subsets of nonmalignant immune cells to proteasome inhibition has implications in noncancerous pathologic states involving antibody production such as lupus [94]. Preclinical data has also suggested that proteasome inhibition may target highly activated lymphocytes [94,95], which may make it attractive as a therapeutic agent for some autoimmune diseases. In addition, unlike conventional T cells, naturally occurring $CD4^+CD25^+$ T regulatory cells are resistant to the proapoptotic effects of bortezomib during in vitro stimulation [96]. Other preclinical models of autoimmune diseases have been shown to be responsive to bortezomib treatment [52,97], and may be correlated to multiple actions of

the drug, including reduction in pro-inflammatory cytokines and/or direct effects on the effector populations.

Based on our studies (W.J.M.) for the prevention of GVHD with bortezomib treatment [95,98,99] (discussed in a subsequent section), we hypothesized that the proteasome inhibitor bortezomib would have activity in T cell-mediated autoimmune diseases. We examined the ability of the drug to prolong the induction and prevent relapses of experimental autoimmune encephalomyelitis (EAE) in SJL/J mice immunized with a neuroantigenic peptide (proteolipid protein, $PLP_{139-152}$), a model of relapsing-remitting multiple sclerosis (MS) in humans. Following immunization with the peptide, mice were treated with 15 μg bortezomib, or with the vehicle. There was no significant difference in the incidence of neurodegenerative disease development in bortezomib-treated mice. However, bortezomib treatment given early in the induction phase of primary EAE delayed the onset and reduced the severity of clinical signs and treatment during first remission prevented recurrence of clinical symptoms of EAE. Additionally, the data indicated that the disease will reoccur after withdrawal of bortezomib therapy (W.J.M., manuscript in preparation).

However, the immunosuppressive activity of bortezomib may also result in potential adverse effects, especially in cancer patients who may already have decreased immune function resulting from the tumor and/or other therapies. Indeed, even though proteasome inhibition may sensitize virally infected lymphocytes to apoptosis [100], bortezomib can induce lytic infection in Epstin-Barr virus (EBV^+) cells [101] and, in immune control of latent disease, may be compromised, which results in viral reactivation, and increased incidence of varicella zoster (VZV) infections have been reported in patients treated with bortezomib [102,103]. However, in situations of adoptive cellular immunotherapy, the effects on target and effector cell populations can be separated by pretreatment with bortezomib followed by cellular immunotherapy to yield effective outcomes. Such immunotherapy may consist of dendritic cell vaccination [87], activated NK cell infusion (NCT00720785), or allogeneic HSCT. Thus, the timing and duration of bortezomib administration may be critical for therapeutically modulating immune responses.

Bortezomib and Hematopoietic Engraftment

Critical to the success of any agent employed after transplantation is the potential impact on engraftment and stem cell function. In preclinical models, there did not appear to be an impact on hematopoietic engraftment following a short, 2-day course of bortezomib at the time of transplant [95]. Time to white blood cell and platelet engraftment were not affected by bortezomib. In addition, donor T cell engraftment was also not affected by bortezomib, with all animals demonstrating >90% T donor cell engraftment [95]. The impact of proteasome inhibition on HSC function has also been studied directly in mice. Stem cell function was unaffected by treatment with bortezomib for 4 21-day cycles, as evaluated by in vitro bone marrow (BM) colony formation. In addition, in vivo repopulation of peripheral blood stem cells (PBSCs) in lethally irradiated mice, after transplantation with BM from bortezomib-treated donors, was equivalent to that of irradiated mice transplanted with untreated donor BM [104]. These findings indicate that bortezomib treatment does not compromise HSC function.

In clinical trials using bortezomib in heavily pretreated patients with MM, thrombocytopenia was noted in regimens where the drug was given more frequently than once per week [105]. When given biweekly, a transient drop in platelets was noted after the administration of each dose of bortezomib. However in these trials, platelet count recovery was prompt, with no increased risk of bleeding complications and rarely required dose reduction or delay in administration of bortezomib [106]. In the autologous transplant context, the effect of bortezomib alone and in combination with other agents on the mobilization and engraftment of HSC has been evaluated in clinical trials involving patients with relapsed/refractory or previously untreated MM. In 7 separate clinical trials incorporating bortezomib with other agents prior to mobilization, autologous stem cell harvesting and engraftment was successful [107-114]. Such therapy was tolerable with toxicities that were manageable and generally similar across studies. Taken together, these studies indicate that proteasome inhibition does not damage the HSC compartment except for a potential transient effect on megakaryocyte precursors.

Bortezomib and Allogeneic HSCT—Preclinical Models

Based on its potential to sensitize tumor cells to allogeneic NK and T cell targeting of donor graft cells, and ability to modulate immune responses, bortezomib is an attractive chemotherapeutic agent to use in combination with allogeneic HSCT, for its potential ability to promote GVT, and to control GVHD responses. We documented that a short course of bortezomib peritransplant reduced the development of acute lethal GVHD in a fully MHC mismatched murine model of allogeneic HSCT [95]. Bortezomib administration to the BM transplant (BMT) recipient was associated with a decrease in nuclear NF-κB expression and a decrease in inflammatory cytokine

expression. These findings suggest that bortezomib may be acting, at least in part, through inhibition of NF-κB activation. All mice in the fully MHC mismatched study eventually succumbed to complications of GVHD. In a model intended to induce less severe GVHD, mice receiving bortezomib had a 100% survival with no animal developing GVHD, whereas mice not receiving bortezomib all succumbed to GVHD prior to day 50 posttransplantation [95].

Although the early addition of bortezomib was successful in reducing the incidence of GVHD, it also preserved GVT responses. This is critical, because many interventions that reduce the incidence of GVHD also lead to a reduction in the GVT effect. Bortezomib has been shown to have antitumor effects both in vitro and in vivo. In the same murine models, tumor-bearing mice that received allogeneic BMT, donor splenocytes, and bortezomib had an improved survival compared with mice receiving transplantation alone [95].

In a note of caution, however, prolonged administration of bortezomib resulted in a GVHD-like lethal toxicity affecting the gastrointestinal tract (GI) of treated animals [99,115]. This toxicity was associated with a rise in the inflammatory cytokines, IL-1β, IL-6, and TNF-α, and with an increase in the expression of TNFR1 in the intestinal tissues of affected animals [99]. The observed toxicity may be because of broader activity of bortezomib beyond inhibition of the NF-κB pathway [116]; as a second and more selective inhibitor, PS-1145, did not result in toxicity in late or extended treated allogeneic HSCT recipient mice [115].

The critical requirement for allogeneic T cells in bortezomib-associated toxicity posttransplant is demonstrated in the lack of toxicity in recipients of allogeneic transplantation with a limited dose of T cells contained within the BM, which is insufficient to induce clinical symptoms of aGVHD. Allogeneic $CD4^+$ T cells were found to be critical for the development of toxicity with bortezomib [98]. Thus, depletion of $CD4^+$ T cells from the graft or administration of enriched $CD8^+$ T cells could allow for prolonged or delayed administration of bortezomib without exacerbation of GVHD [98]. Importantly, these studies demonstrated that continuous administration of bortezomib postallogeneic HSCT can enhance $CD8^+$ T cell-mediated GVT, resulting in prolonged survival of tumor-bearing mice [98]. The allogeneic $CD4^+$ T cell-associated toxicity after bortezomib administration is dependent on TNF-α expression in this population of cells [98]. Furthermore, TNF-α has been shown to be important in $CD4^+$ T cell-mediated GVHD. In acute murine GVHD models, bortezomib apparently heightens sensitivity of host GI tissues to TNF-α-dependent $CD4^+$ T cell attack [98].

Proteasome Inhibition and Adoptive Immunotherapy

As noted earlier, tumor cells can be sensitized to killing by NK cells [82,83,85] and T cells [85] through death receptor-mediated pathways, that can be enhanced by proteasome inhibition. Of all the TNF receptor superfamily members that utilize death domains and induce apoptosis, it is sensitivity to TRAIL/TRAIL death receptor (DR)-mediated killing following bortezomib treatment that has been shown to be acting in a broad variety of lymphohematopoietic tumors and carcinomas [73,83,84,117-122]. This activity may be mediated, in part, through the upregulation of the TRAIL receptor, DR5 on the tumor cell surface [73,84], as a consequence of DR5 mRNA stabilization [123], and as an increase in proapoptotic proteins within the cell [73,124,125], although the precise mechanisms appear to differ between different tumor types. Recombinant TRAIL and agonist antibodies to TRAIL receptors DR4 or DR5 have been evaluated in phase Zweegman et al is [128]. I and II clinical trials (reviewed in Zweegman et al. [128]).

Another therapeutic approach combines bortezomib with adoptive immunotherapy to achieve antitumor responses that may be greater than can be achieved than reliance on a single death receptor pathway. Bortezomib administration postallogeneic HSCT is predicated in part on the notion that continuous administration of the proteasome inhibitor will sensitize residual tumor cells to killing by donor-derived T cells and NK cells [83,98]. These cells can kill through a broad repertoire of cytotoxic molecules (Figure 1). Adoptive cellular therapy with ex vivo expanded human autologous NK cells after presensitization of tumor cells with bortezomib [83,86,127] has also been explored in preclinical models where the combination of bortezomib sensitization and IL-2 expanded NK cells has shown efficacy in both in vitro and in vivo mouse tumor models [82,127]. This strategy is currently being evaluated in a phase I trial (NCT00720785; http://clinicaltrials.gov) of escalating doses of adoptively infused ex vivo expanded autologous NK cells in patients with treatment refractory metastatic solid tumors or hematologic malignancies, which are sensitized to NK cell cytotoxicity using bortezomib. The combined use of bortezomib and NK infusions are also under investigation in a clinical trial that will assess myeloid recovery and incidence of aGVHD as its primary measures. In this phase I/II trial, donor NK cell infusions and rhIL-2 are administered as part of a reduced intensity conditioning (RIC) preparative regimen consisting of bortezomib, cyclophosphamide (Cy), fludarabine (Flu), antithymocyte globulin (ATG), and total body irradiation (TBI) prior to haploidentical allogeneic HSCT, for patients with myelogenous leukemia or myelodysplastic syndromes

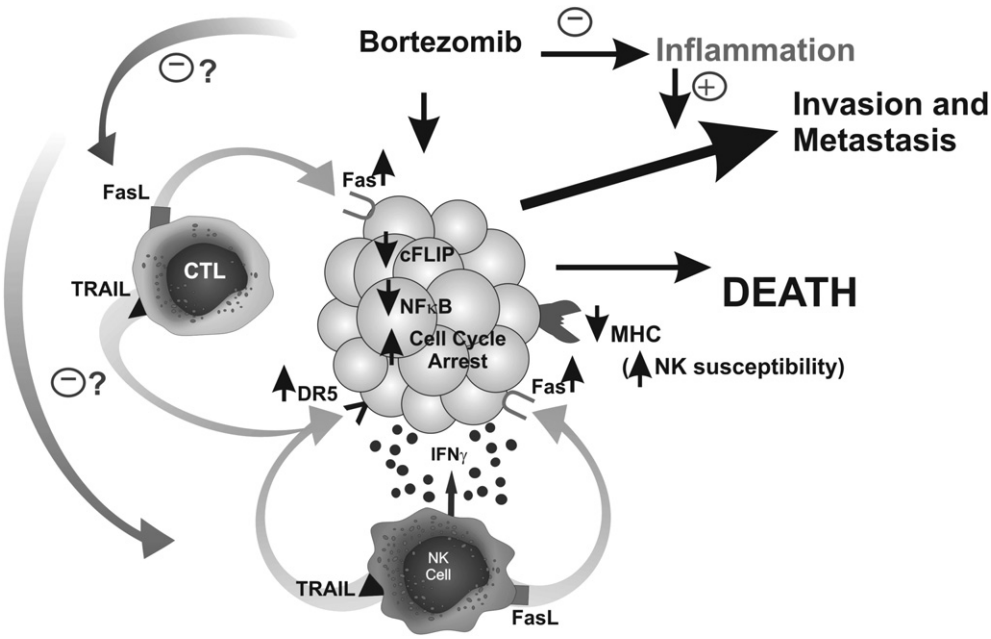

Figure 1. Potential mode of action in bortezomib mediated antitumor responses. Bortezomib may have a negative impact on the growth and spread of cancer cells through multiple mechanisms including direct induction of apoptosis, sensitization to killing by $CD8^+$ cytotoxic T cells and NK cells, and through reduction of inflammation resulting in decreased metastasis.

(MDS) who have been deemed unsuitable for fully matched MA transplantation (NCT00303667; http://clinicaltrials.gov).

Proteasome Inhibition and Clinical aGVHD

Preliminary clinical data indicate that bortezomib administration after allogeneic HSCT can control GVHD [128]. In a retrospective analysis involving 9 patients with MM relapsed after allogeneic HSCT, treatment with bortezomib was effective in MM control, with 2 very good partial responses (VGPR) and 4 partial responses (PR) [129]. None of the patients in this small cohort developed GVHD, suggesting that proteasome inhibition after allogeneic HSCT did not exacerbate GVHD in humans. Bortezomib was reasonably well tolerated; grade 3 and 4 toxicities noted included grade 4 thrombocytopenia, grade 3 fatigue, and grade 3 diarrhea and hypotension. In a larger retrospective study of 3 European centers, administration of bortezomib as salvage for relapse or progression of MM after RIC allogeneic HSCT did not result in worsening of GVHD symptoms. In addition, 27 of 37 patients had an objective response to bortezomib therapy [130]. In both studies, bortezomib was reasonably well tolerated in the clinical allogeneic HSCT context [129,130].

More direct evidence for GVHD control was reported in a small series of patients with MM relapsed after nonmyeloablative (NMA) allogeneic HSCT that was refractory to donor lymphocyte infusion (DLI) who were subsequently treated with bortezomib. Eleven patients received biweekly bortezomib dosed at 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days (2 patients received bortezomib combined with thalidomide) for a median of 6 cycles, and 10 had a clinical responses [131]. The major reported toxicity was neuropathy grade 2. No GVHD was noted in patients receiving bortezomib, despite the documented strong correlation between clinical response to donor lymphocyte infusion (DLI) and GVHD [132]. In a more recent report, a small cohort of patients receiving DLI and bortezomib was well tolerated. Of the 8 patients receiving a median of 4 cycles of bortezomib, no grade III-IV toxicities were observed although 1 patient developed a Herpes zoster virus (HZV) infection [133].

Prospective trials evaluating proteasome inhibition for GVHD control are underway. A trial combining bortezomib plus tacrolimus and methotrexate (MTX) for GVHD prophylaxis after RIC HLA-mismatched unrelated donor allogeneic HSCT recently reported preliminary data in abstract form (NCT00369226; http://clinicaltrials.gov). Administration of bortezomib dosed at 1.3 mg/m^2 on days +1, +4, and +7, was found to be safe and efficacious. No neurotoxicity or intestinal toxicity was noted, and neutrophil and platelet engraftment was prompt. Grade II-IV aGVHD occurred in 2 of 17 evaluable patients, for a 180 day cumulative incidence of 14%, with relapse or death as a competing risk [134]. Additional trials of proteasome inhibition for GVHD prophylaxis in the MA context (NCT00670423), and for therapy of steroid-refractory aGVHD (NCT00408928) are ongoing.

Thus, bortezomib therapy, either early or late after allogeneic HSCT appears reasonably well tolerated in

human studies reported thus far. Similar to observations in the preclinical mouse models, the timing and duration of peritransplant bortezomib therapy, and its interaction with conditioning therapy, likely play an important role in determining its toxicity and efficacy in humans. For instance, consistent with the colonic toxicity noted in mice after prolonged bortezomib exposure posttransplant [99], in an allogeneic HSCT trial of 11 patients with refractory/relapsed acute myelogenous leukemia (AML) or MM at the M.D. Anderson Cancer Center receiving allogeneic HSCT after M conditioning, the cohort receiving bortezomib (0.7 mg/m^2) on days +1, +5, +9, and +12 was found to have unacceptable grade 4 intestinal toxicity (resulting in study closure), which was not seen in the cohort receiving bortezomib prestem cell infusion (on days −12, −9, −6, and −3) (S. Giralt, personal communication). Other ongoing trials at M.D. Anderson Cancer Center, evaluating bortezomib prestem cell infusion, plus rituximab and BEAM conditioning for lymphoid malignancy allogeneic HSCT, have not encountered such toxicity (NCT00439556; http://clinicaltrials.gov), hig-hlighting the importance of bortezomib timing and concomitant therapies in study outcomes.

Proteasome Inhibition and Clinical cGVHD

In the cGVHD setting, clinical reports of bortezomib's activity in the disease have preceded any preclinical studies. Published reports have highlighted the safety of bortezomib and its ability to control active cGVHD in myeloma patients relapsed after allogeneic HSCT. One case report describes a patient with medullary and extramedullary myeloma relapse postallogeneic HSCT, which was refractory to local X-ray radiation therapy and 3 doses of DLI [135]. The MM was treated with 2 cycles of bortezomib at a dose of 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days, with disappearance of the extramedullary mass and discontinuation of bortezomib. The patient subsequently developed mucocutaneous lichen planus and biopsy-proven hepatic cGVHD, and was restarted on bortezomib monotherapy for 8 more cycles. This resulted in an excellent clinical response, including normalization of liver function tests and disappearance of oral cGVHD lesions. However, grade 2 neuropathy was noted after extended use and necessitated interruption of bortezomib after 6 cycles. A larger series described the use of bortezomib in 8 patients with MM relapsed after allogeneic HSCT [136]. Four patients had active cGVHD (2 steroid refractory) at the time of starting bortezomib therapy, including 3 patients with severe punctuate keratopathy (ocular cGVHD). They received a median 6 cycles of bortezomib (range: 3-12), dosed at 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days. Interestingly, in all 4 patients cGVHD was significantly improved, and ocular cGVHD remained in remission at a median of 150 days (range: 120-333 days) after discontinuation of bortezomib. Improvement in ocular cGVHD is remarkable, as it is typically very resistant to conventional therapy, including steroids [3,137]. Grade 3-4 toxicities involved thrombocytopenia (50%), neuropathy (25%), leukopenia (12%), and GI toxicity (50%).

Some toxicity was also noted in a study evaluating biweekly administration of bortezomib to improve disease-free survival (DFS) in patients without evidence of relapsed or progressive MM after allogeneic HSCT [138]. Eighteen patients received bortezomib at a dose of 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days for 2 to 4 cycles. Nine of the 18 patients received concomitant cyclosporine (CsA), and 2 patients received additional low-dose thalidomide. Grade 3-4 toxicities observed in these patients included thrombocytopenia (50%), leukopenia (17%), and neuropathy (17%). Significant neuropathy was only observed in patients receiving concomitant CsA therapy (3 versus 0; $P = .06$). Of note, 2 patients with preexisting cGVHD experienced mild progression involving the mouth or skin that did not require any systemic immunosuppressive therapy. In contrast, toxicity was mild in a larger study of 37 patients who received biweekly bortezomib for MM relapsed after RIC allogeneic HSCT, that was typically dosed at 1.3 mg/m^2 on days 1, 4, 8, and 11 every 21 days for a median of 6 cycles (range: 1-15) [130]. Only grade 1-2 toxicities were observed, which included thrombocytopenia (24%) and peripheral neuropathy (35%), with a median onset of 83 days after initiation of therapy. Significant myeloma control was noted. Additionally, 2 of the 3 patients with preexisting extensive cGVHD at the start of therapy experienced significant improvement. A prospective clinical trial of bortezomib plus prednisone for initial therapy of newly diagnosed cGVHD is ongoing (NCT00815919; http://clinicaltrials.gov).

Based on our limited understanding of the pathophysiology of cGVHD and the therapeutic targets of bortezomib treatment in other preclinical models such as aGVHD [95,115], EAE, and lupus-like nephritis [94], it can be postulated that proteasome inhibition may act on established cGVHD. This is because proteasome inhibition eliminates alloreactive plasma cells and alloreactive T cells. Furthermore, it interferes with DC function and reduces inflammatory cytokine production either by eliminating the cytokine-secreting cell or by inhibiting its production through the down-modulation of NF-κB-dependent transcription. Preclinical studies will provide a better understanding of the mechanism of action, which may lead to optimized design of therapeutic protocols with this compound.

FUTURE DIRECTIONS

Molecular targeting, using small molecules, has emerged as an effective means to target neoplastic

cells. Proteasome inhibition using bortezomib is currently approved as a front-line treatment for multiple myeloma and for the treatment of relapsed mantle cell lymphoma. Moving beyond its currently approved indications, proteasome inhibitors act on many cellular pathways to exert effects on neoplastic cells. Interference with the NF-κB pathway may be critical for many of the immunomodulatory properties associated with this class of drugs The use of proteasome inhibitors in the context of allogeneic HSCT is currently under intensive investigation, because of its potential to provide both direct and indirect antitumor effects after allogeneic HSCT, as well as exerting anti-inflammatory effects that may further improve GVHD control. This is an exciting new area of investigation of combining cellular immune therapies with molecularly targeted novel agents, with a goal to control GVHD while preserving GVT responses. The outcome of clinical trials evaluating bortezomib in the context of allogeneic HSCT and other adoptive immunocellular therapies is eagerly awaited. However, the potential effect of these agents on immune reconstitution (or the immunotherapy), aGVHD and cGVHD, and GVT responses after allogeneic HSCT is still unresolved, and appropriate caution needs to be exercised.

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