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Brief Articles

Short Course of Post-Transplantation Cyclophosphamide and Bortezomib for Graft-versus-Host Disease Prevention after Allogeneic Peripheral Blood Stem Cell Transplantation Is Feasible and Yields Favorable Results: A Phase I Study

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A B S T R A C T

An effective graft-versus-host disease (GVHD) preventative approach that preserves the graft-versus-tumor effect after allogeneic hematopoietic stem cell transplantation (HSCT) remains elusive. Standard GVHD prophylactic regimens suppress T cells indiscriminately and are suboptimal. Conversely, post-transplantation high-dose cyclophosphamide selectively destroys proliferating alloreactive T cells, allows the expansion of regulatory T cells, and induces long-lasting clonal deletion of intrathymic antihost T cells. It has been successfully used to prevent GVHD after allogeneic HSCT. Bortezomib has antitumor activity on a variety of hematological malignancies and exhibits a number of favorable immunomodulatory effects that include inhibition of dendritic cells. Therefore, an approach that combines post-transplantation cyclophosphamide and bortezomib seems attractive. Herein, we report the results of a phase I study examining the feasibility and safety of high-dose post-transplantation cyclophosphamide in combination with bortezomib in patients undergoing allogeneic peripheral blood HSCT from matched siblings or unrelated donors after reduced-intensity conditioning. Cyclophosphamide was given at a fixed dose (50 mg/kg on days +3 and +4). Bortezomib dose was started at .7 mg/m^2, escalated up to 1.3 mg/m^2, and was administered on days 0 and +3. Patients receiving grafts from unrelated donors also received rabbit antithymocyte globulin. The combination was well tolerated and allowed prompt engraftment in all patients. The incidences of acute GVHD grades II to IV and grades III and IV were 20% and 6.7%, respectively. With a median follow-up of 9.1 months (range, 4.3 to 26.7), treatment-related mortality was 13.5% with predicted 2-year disease-free survival and overall survival of 55.7% and 68%, respectively. The study suggests that the combination of post-transplantation cyclophosphamide and bortezomib is feasible and may offer an effective and practical GVHD prophylactic regimen. The combination, therefore, merits further examination.

INTRODUCTION

Despite the routine use of immunosuppressive drugs targeting T cells, graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT) remains a significant cause of morbidity and mortality limiting the utility and wide applicability of this potentially curative procedure [1,2]. Two large single-institution and Center for International Blood and Marrow Transplant Research reports suggest that the incidence of acute and chronic GVHD has increased in recent years [3,4]. Additionally, the current standard regimens impair the graft-versus-tumor (GVT) effect, delay immune reconstitution, and are burdensome [5,6].

Cyclophosphamide, administered in high doses early after transplantation, allows for long-term tolerance by inducing selective depletion of proliferating host-reactive T cells, promoting regulatory T cells, and producing long-lasting intrathymic clonal deletion of antihost T cells (reviewed by Luznik et al.) [7]. When used alone after matched related and unrelated donor bone marrow HSCT, cyclophosphamide

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reduces the incidence of chronic but not acute GVHD without impairing engraftment (reviewed in [8]). Furthermore, its selective activity targeting proliferating rather than resting cells suggests that it may preserve the GVT effect and permit rapid immune reconstitution [9].

Bortezomib occupies proteasomal proteolytic sites, disrupts mitochondrial function, and produces endoplasmic stress in dendritic antigen-presenting cells and T cells [10-12]. Consequently, cells treated with bortezomib exhibit a decreased expression of maturation and functional markers, reduced ability to produce proinflammatory cytokines, impaired mobilization, cell cycle arrest, and apoptosis [11,13-16]. Other beneficial characteristics of bortezomib include a more pronounced proapoptotic activity on alloreactive T cells [13] and an ability to preserve regulatory T cells and foster the emergence of a distinct population of suppressor T cells [17]. Several mouse models and clinical trials have shown that bortezomib is active in preventing GVHD when given as a short course after transplantation [18-21]. Bortezomib is also active in a variety of hematological malignancies and enhances natural killer cell–induced tumor cytotoxicity [22,23].

Therefore, we wanted to explore the feasibility and safety of combined high-dose post-transplantation cyclophosphamide with bortezomib after allogeneic HSCT. Our aim was to concomitantly target dendritic and T cells. Herein, we report the results of this phase I study.

MATERIALS AND METHODS

Eligibility

Patients with hematological malignancies undergoing allogeneic peripheral blood HSCT from related or unrelated donors after reduced-intensity conditioning were considered. Donors had to be allele-matched to the recipient at HLA loci A, B, C, and DRB1. Inclusion criteria included age $\geq$ 18 years, Karnofsky performance status $\geq$ 70%, creatinine clearance $>$ 40 mL/minute/1.73 m^2, total bilirubin $<$ 1.5 mg/dL, transaminases $<$ 2 times the upper limit of normal, left ventricular ejection fraction $>$ 40%, corrected carbon monoxide diffusing capacity $>$ 50%, negative pregnancy test, and negative human immunodeficiency virus test. Eligible patients had to be able to provide informed consent, agree to prevent pregnancy for at least 30 days after the last dose of bortezomib, and show no evidence of progressive bacterial, viral, or fungal infection despite adequate treatment before the initiation of the preparative regimen. Exclusion criteria included peripheral neuropathy $\geq$ grade 2, uncontrolled angina, myocardial infarction within 6 months from enrollment, electrocardiographic evidence of acute ischemia, severe uncontrolled ventricular arrhythmias, active conduction system abnormalities, a New York Heart Association class III or IV heart failure, serious medical or psychiatric illness that could interfere with participation, and another malignancy within 3 years of enrollment (with the exception of a completely resected basal or squamous cell carcinoma of the skin, treated in situ malignancy, or low-risk curatively treated prostate cancer). Patients with hypersensitivity to bortezomib, boron, or mannitol were also excluded. The study was approved by the institutional review boards (Roger Williams Medical Center, Providence, RI and Spectrum Health, Grand Rapids, MI) in accordance with federal regulations and the Declaration of Helsinki. All patients signed written informed consent before enrollment in the study. This trial was registered at ClinicalTrials.gov, identifier: NCT01860170.

Conditioning Regimen and Supportive Care

As shown in Figure 1, patients received a reduced-intensity conditioning regimen consisting of fludarabine 30 mg/m^2/day (days −7 to −2) and busulfan (.8 mg/kg) every 6 hours × 8 doses for a total of 6.4 mg/kg (days −3 to −2). Patients receiving grafts from unrelated donors also received rabbit antithymocyte globulin (rATG) (Thymoglobulin, Sanofi, Laval, Canada) 5 or 8 mg/kg cumulative dose per institutional practices. The first 4 patients received 2 mg/kg/day given on days −4 to −1. The remaining 4 patients received 1 mg/kg, 1.5 mg/kg, and 2.5 mg/kg on days −4, −3, and −2, respectively. Each rATG dose was rounded to the nearest 25 mg vial. The graft source was filgrastim-mobilized peripheral blood stem cells with a required minimum dose of $CD34^+$ cells of 2×10^6/kg and no upper limit. The study was designed to enroll patients in a standard 3 plus 3 design. Cyclophosphamide was administered at a fixed dose of 50 mg/kg/day i.v. with forced hydration on days +3 and +4. Bortezomib was given at escalating doses in 3 consecutive cohorts (.7, 1, and 1.3 mg/m^2) administered i.v. 6 hours after graft infusion and 72 hours thereafter if no dose limiting toxicity (DLT) occurred in $\geq$ 1 out of 3 or 2 out of 6 patients. *DLT* was defined as any grade 3 or 4 nonhematologic toxicity, grade $\geq$ 2 hyperbilirubinemia, or graft failure. Antimicrobial prophylaxis was administered per institutional standard practices. Acyclovir was started on day +5 in the first 6 patients and with the start of conditioning for the remaining patients. All patients received filgrastim 5 μg/kg starting on day +7. Quantitative cytomegalovirus (CMV) PCR was monitored weekly starting at engraftment and continued until at least day +100. Epstein-Barr virus and adenovirus PCR were monitored in patients receiving rATG.

Adverse Events and Patient Monitoring

Patients were followed prospectively for adverse events from the initial dose of bortezomib and until 30 days from the last dose. All adverse side effects were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. *Neutrophil engraftment* was defined as achieving an absolute neutrophil count $\geq .5 \times 10^9$/L for 3 consecutive measurements on different days. The first of the 3 days was considered the day of neutrophil engraftment. *Platelet engraftment* was defined as a platelet count $\geq 20 \times 10^9$/L for 3 consecutive days without platelet transfusion for 7 days. The first of the 3 days was considered the day of platelet engraftment. Donor chimerism was first assessed at neutrophil engraftment. *Graft failure* was defined as lack of neutrophil engraftment by day +22 and absence of donor chimerism $>$ 50% by day +45. *Secondary graft failure* was defined as persistent decline in neutrophil count to $< .5 \times 10^9$/L and donor chimerism $<$ 5% in the absence of disease relapse. Patients were assessed for both acute and chronic GVHD. The clinical diagnosis of GVHD was confirmed by histology whenever possible. The first day of acute or chronic GVHD of a certain grade was used to calculate the cumulative incidence for that grade. Acute GVHD was graded according to the modified Keystone criteria. Upper gastrointestinal GVHD was considered stage 1. Chronic GVHD grading was based on the National Institute of Health criteria.

Study Design and Statistical Analysis

The primary objective of this phase I study was to determine the feasibility and safety of bortezomib administered after transplantation in conjunction with a fixed high-dose cyclophosphamide as GVHD prophylaxis. Secondary objectives were to determine the incidence of graft failure, acute and chronic GVHD, treatment-related mortality (TRM), progression-free survival (PFS), and overall survival (OS). TRM was defined as death without evidence of recurrent disease. PFS and OS were estimated from day 0 according to the Kaplan-Meier method.

RESULTS

Patient Characteristics

Patient characteristics and outcomes are summarized in Table 1. Fifteen patients were enrolled: 3 in each of cohorts 1

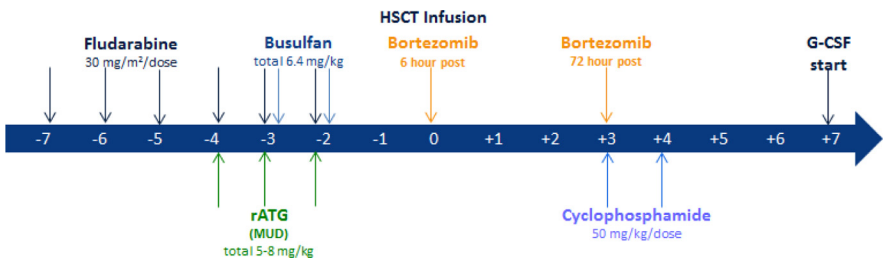

Figure 1. Treatment scheme. MUD indicates matched unrelated donor.

Table 1
Patient Characteristics and Outcomes

Patient ID	Age	Gender	Diagnosis	DRI	KS	Disease Status at Transplantation	CD34 × 10^6/kg	Bor Dose (mg/m^2)	Donor	CMV Status D/R	Infectious Complications	Acute GVHD				Chronic GVHD	Secondary Systemic Immunosuppression	Follow Up; Current Status	Cause of Death
												Skin Stage	GI Stage	Liver Stage	Overall Grade				
1	56	F	CLL	low	90	PR1	8.24	.7	MRD	+/+		0	0	0	0	None		26.7 m; NED	
2	65	F	MDS	high	90	PR1	6.19	.7	MUD	−/−		1	0	0	I	None	Steroids low dose, short course	30 m; NED	
3	60	F	FL	low	80	PR4	10.1	.7	MUD	+/+	CMV reactivation, CNS toxoplasmosis, and G-R HSV	2	0	0	I	Moderate	Steroids low dose, short course for acute and prednisone and MMF for chronic GVHD	24.7 m; NED	
4	59	F	AML	high	80	CR1	3.57	1.0	MUD	+/Unk	CMV reactivation, G-R HSV, and RSV and candida glabrata pneumonitis	0	0	0	0	None		Deceased at 5 m	RSV and candida glabrata pneumonitis
5	62	F	MDS	int	80	PR1	3.63	1.0	MRD	+/+		0	0	0	0	Severe	Steroids for chronic GVHD	Deceased at 6.7 m	Acute sepsis
6	47	M	MM	int	80	PD (4 lines of therapy)	13.32	1.0	MUD	−/+	EBV reactivation	2	0	0	I	None	Steroids low dose, short course	Progression at 3.9 m; deceased at 9.6 m	Disease progression
7	51	M	CLL	low	90	PR3	2.76	1.3	MRD	−/−		1	0	0	I	None		23.9 m; NED	
8	54	M	MDS	high	100	CR1	7.11	1.3	MRD	+/+	CMV reactivation	1	0	0	I	None		Progression at 10.7 m; alive at 14.5 m	
9	55	M	CLL	low	90	PR3	5.2	1.3	MRD	+/+	CMV reactivation	3	0	0	II	None	Steroids	10.6 m; NED	
10	65	F	MDS	very high	80	PD (1 line of therapy)	2.69	1.3	MRD	+/−	CMV and EBV reactivation	2	0	0	I	Severe	Steroids and CSA for chronic GVHD	Progression at 6.8 m; deceased at 7.4 m	Disease progression
11	57	M	AML	low	100	CR2	4.55	1.3	MUD	+/+	CMV and EBV reactivation	0	0	0	0	None		Progression at 9.1 m; alive at 9.1 m	
12	43	F	AML	low	100	CR2	5	1.3	MUD	+/−	BK virus hemorrhagic cystitis	0	0	0	0	*		7.1 m; NED	
13	43	M	DLBCL	int	90	PR3	5.01	1.3	MUD	+/−	CMV reactivation	3	0	0	II	*	Steroids	Progression at 2.6 m; alive at 5.9 m	
14	66	M	MDS	high	90	PD (2 lines of therapy)	5	1.3	MRD	+/−	CMV reactivation	0	3	0	III	*	Steroids and CSA for acute GVHD	5 m; NED	
15	37	M	AML	int	90	CR1	7.12	1.3	MUD	+/+		2	0	0	I	*	Steroids low dose, short course	4.3 m; NED	

DRI indicates disease risk index; KS, Karnofsky status; Bor, bortezomib; D, donor; R, recipient; GI, gastrointestinal; F, female; CLL, chronic lymphocytic leukemia; PR, partial remission; MRD, matched related donor; NED, no evidence of disease; MUD, matched unrelated donor; FL, follicular lymphoma; CNS, central nervous system; G-R HSV, genito-rectal herpes; MMF, mycophenolate mofetil; AML, acute myelogenous leukemia; CR, complete remission; Unk, unknown; RSV, respiratory syncytial virus; M, male; int, intermediate; PD, progressive disease; EBV, Epstein-Barr virus; DLBCL, diffuse large B cell lymphoma; CSA, cyclosporine A.

and 2 and 9 in cohort 3, as no DLT occurred in any patient. The first 7 patients were enrolled at Roger Williams Medical Center and the remaining 8 at Spectrum Health. The median age was 56 years (range, 37 to 66). There were 7 females and 8 males. Four patients had acute myelogenous leukemia, 5 myelodysplastic syndrome (MDS), 3 chronic lymphocytic leukemia, and 1 each of follicular lymphoma, multiple myeloma (MM), and diffuse large B cell lymphoma. Disease risk index was low in 6, intermediate in 4, and high or very high in 5 patients. At the initiation of conditioning, 2 patients with acute myelogenous leukemia were in first and 2 in second complete remission. Four of 5 patients with MDS had active disease. The patient with MM had progressive disease after 2 autologous transplantations. The patient with diffuse large B cell lymphoma had relapsed within 1 year after autologous transplantation but remained chemo-sensitive. All the remaining patients were in partial remission. All patients received filgrastim-mobilized peripheral blood stem cells. The infused median dose of $CD34^+$ cells was 5.01×10^6/kg (range, 2.69 to 13.32). All patients completed the planned treatment. There was 1 protocol violation with a patient receiving a 5-day course of steroids for a biopsy-proven drug eruption.

Engraftment and GVHD

Engraftment was prompt in all patients. Median time to neutrophil engraftment was 16 days (range, 13 to 23). Two patients died before achieving platelet engraftment. The first patient had acyclovir-resistant herpes genitalis and CMV reactivation requiring protracted therapy with foscarnet and died on day +150 from respiratory syncytial virus and candida glabrata pneumonitis. The second patient died on day +200 because of acute sepsis in the setting of chronic GVHD. Median time to platelet recovery in the remaining patients was 28 days (range, 15 to 38). All patients achieved full donor chimerism by day +28, except for 1 patient who had residual chronic lymphocytic leukemia at engraftment and did not reach full donor chimerism until day +118 after developing acute GVHD. No patient developed secondary graft failure.

The overall incidence of acute GVHD was 67% for all grades, 20% grades II to IV, and 6.7% for grades III and IV. There was no grade IV GVHD and only 1 patient developed visceral acute GVHD. Overall, 7 patients received systemic steroids for acute GVHD; 4 of them received abbreviated course of prednisone $<$ .5 mg/kg for grade I disease involving the face. Three of 12 (25%) patients with follow-up $>$ 6 months developed chronic GVHD; 1 moderate and 2 severe. Overall, 6 patients (40%) have not required any secondary immunosuppressive therapy thus far.

Nonhematologic Toxicity and Adverse Events

Two patients developed extensive herpetic genito-rectal ulcers; 1 had prior history of recurrent flares with acyclovir-resistant disease. When institutional guidelines were changed to start acyclovir at the beginning of conditioning, as opposed to day +5, no other cases were noted. Eight patients developed CMV reactivation and received preemptive therapy. No CMV disease occurred. Three patients developed Epstein-Barr virus reactivation, of whom 2 required preemptive therapy with rituximab. One patient developed BK virus–induced hematuria and 1 patient developed central nervous system toxoplasmosis. One patient, mentioned above, developed respiratory syncytial virus and candida glabrata pneumonia. All other side effects were grade 1 or 2. The most common side effects were gastrointestinal and transient elevation of liver function tests. One patient experienced rigors after cyclophosphamide infusion. Additional events included syncope (n = 1), orthostatic hypotension (n = 2), and venous thromboembolism (n = 2).

Relapse and Mortality

There was no mortality by day +100. With a median follow-up of 9.1 months (range, 4.3 to 26.7), 2 (13.5%) treatment-related deaths occurred on days +150 and +200. The patient with MM and a patient with high-risk MDS died because of progressive disease. Overall, disease relapse occurred in 5 patients (33%) on days +79, +118, +205, +271, and +321. The 2-year predicted PFS and OS were 55.7% and 68%, respectively.

DISCUSSION

An optimal GVHD prevention regimen must be effective, permit rapid immune reconstitution, and preserve the GVT effect. The regimen should also be well tolerated and not burdensome to use. The current pharmacologic prophylaxis, employing different combinations of methotrexate, mycophenolic acid, calcineurin inhibitors, mammalian target of rapamycin (mTor) inhibitors, and antithymocyte globulin all aim to indiscriminately inhibit or eliminate T cells and, therefore, fall short of meeting the above requisites. No specific combination has proved superior [24]. A recent study comparing the commonly used combination of tacrolimus and methotrexate to a novel duplet of tacrolimus and sirolimus after matched sibling donor HSCT failed to demonstrate any significant improvement. The incidence of grade II to IV acute and chronic GVHD was 34% and 43% in the control arm and 26% and 54% in the study arm, respectively [25]. Furthermore, although oral mucositis was decreased, the experimental combination was associated with higher incidence of thrombotic microangiopathy and hepatic sinusoidal occlusive syndrome.

Luznik et al. introduced cyclophosphamide administered in high doses in the early post-transplantation period as single-agent prophylaxis in patients undergoing matched related or unrelated donor transplantation after myeloablative conditioning [26]. As opposed to pan T cell suppression, cyclophosphamide activity seems selective to donor alloreactive cells [9]. The original results were recently confirmed by Kanakry et al. in a multi-institutional trial using bone marrow as the source of stem cells. The incidences of grade II to IV and grade III and IV acute GVHD remained significant, however, at 50% and 15%, respectively [27]. The incidence of chronic GVHD was more favorable (14%). In an attempt to further improve the results, Solomon et al. added sirolimus to post-transplantation cyclophosphamide for patients receiving myeloablative conditioning followed by peripheral blood HSCT from matched related or unrelated donors [28]. The incidences of acute grade II to IV GVHD and chronic GVHD were 46% and 31%, respectively.

Dendritic cells (DC) play a pivotal role in the early development of GVHD and seem to be an ideal target for GVHD prevention. Bortezomib has been shown to inhibit DC maturation and function and possesses a number of other favorable immunomodulatory effects (reviewed by Mohty et al.) [29]. Koreth et al. studied bortezomib in combination with methotrexate and tacrolimus in a high-risk group receiving mismatched unrelated donor transplants [21]. The incidences of acute grade II to IV and chronic GVHD were 22%

and 29%, respectively. Immune reconstitution was also enhanced in comparison to historical control [21].

In the current study, we aimed to explore the feasibility of the addition of bortezomib to a platform of post-transplantation cyclophosphamide, a combination that selectively targets alloreactive T cells and DC. Because we used a reduced-intensity conditioning and peripheral blood stem cells, we opted to add rATG in patients receiving grafts from unrelated donors as originally described by Slavin et al. [30]. We demonstrated that the combination does not impair engraftment. All but 1 patient with residual disease achieved full donor chimerism by day +28. Accounting for the use of peripheral blood as opposed to bone marrow grafts, the median duration to neutrophil and platelet engraftment was comparable to the study by Kanakry et al., where the median times to neutrophil and platelet engraftment were 21 (range, 15 to 42) and 24 days (range, 12 to 65), respectively [27]. In the study by Solomon et al., with peripheral blood as the source of grafts, the median times to neutrophil and platelet engraftment were 15 (range, 13 to 28) and 30 days (range, 16 to 164), respectively [28]. Kanakry et al. reported primary graft failure in 5.4% of patients and secondary graft failure in 2.2% [27]. There was no primary or secondary graft failure encountered thus far in our study. The toxicity of our regimen was also acceptable. As mentioned, there was no grade 3 or greater nonhematologic toxicity. The incidence of infectious complications was also acceptable. Despite frequent CMV reactivation, there was no CMV disease. The primary endpoint of this phase I trial was not to evaluate the efficacy of the combination. Nevertheless, the incidence of grade II to IV acute GVHD of 20% and grade III and IV of 6.6% is encouraging. The incidence of gastrointestinal and hepatic acute GVHD was notably low. With a median follow-up of 9.1 months (range, 4.3 to 26.7) the TRM and the predicted 2-year PFS and OS were within the expected range.

Many questions remain to be addressed. Studies in mice suggest that prolonged administration of bortezomib after allograft is associated with increased incidence of GVHD-dependent gastrointestinal toxicity [31]. A recent study showed that this may be due to paradoxical increase in the production of interleukin-1β (IL-1β) by DC [32]. Although incubating DC with bortezomib before immunogenic stimulation decreases IL-1β production, the addition of bortezomib to already stimulated cells has paradoxical effect. Koreth et al. administered the last dose of bortezomib on day +7 and did not report increased incidence of acute GVHD [21]. However, based on the above concern, we elected to administer the last injection on day +3. Finally, the fact that cyclophosphamide activity requires active proliferation of alloreactive T cells must also be taken into account when using it as a platform in combination with other drugs that may potentially impede T cell proliferation and could potentially abrogate cyclophosphamide activity. We thought that the effect of bortezomib on DC would likely be incomplete and that the early proliferation of T cells would not be entirely prevented. Consequently, we did not delay the administration of the first dose of bortezomib until after cyclophosphamide. Furthermore, we have previously reported that the combination of post-transplantation cyclophosphamide and bortezomib decreases donor DC-induced T cell proliferation in mixed lymphocyte reaction and that this effect is detectable up to day +21 [33]. This suggests that the combination efficiently deletes T cells that are capable of proliferating in response to host antigens. We also felt that the delayed effects of cyclophosphamide on intrathymic host-reactive T cells and regulatory T cells are equally important mechanisms of action of cyclophosphamide and are unlikely to be affected by the administration of bortezomib. Future studies must examine whether the early use of bortezomib before graft infusion can render its prolonged administration safe and allow exploiting its antitumor activity to reduce relapse [23]. The optimal schedule and timing of bortezomib administration in relation to cyclophosphamide must also be studied.

Like post-transplantation cyclophosphamide alone, our regimen allows patients to complete GVHD prevention by day +4 and alleviates the need for patients' strict compliance, concerns about drug interaction, and burden of blood level monitoring when standard pharmacologic agents are used. The regimen might also be useful in patients with renal failure where the use of calcineurin inhibitors has been associated with dismal outcomes [34].

Our study has several limitations. Our patient population is small and the diseases treated and graft source were heterogeneous. Moreover, we need to be cautious when we compare our results to those by Luznik et al. [26] and Kankary et al. [27]. Those studies used myeloablative conditioning and their GVHD prophylaxis did not include rATG. The addition of rATG in our patients who received grafts from unrelated donors (8 of 15) raises ambiguity as to the degree of contribution of bortezomib in the prevention of GVHD. Our study was designed as a feasibility trial and, therefore, the small number of patients enrolled and short follow-up preclude any conclusion with regard to the incidence of GVHD, disease relapse, or survival.

In summary, we have demonstrated that the addition of bortezomib to post-transplantation cyclophosphamide is feasible and well tolerated. The data need to be confirmed in a larger study and several questions remain to be answered. To that end, the study has been extended into a phase II trial.

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