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Feasibility and Efficacy of Partially Replacing Post-Transplantation Cyclophosphamide with Bendamustine in Pediatric and Young Adult Patients Undergoing Haploidentical Bone Marrow Transplantation

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A B S T R A C T

Post-transplantation cyclophosphamide (PT-CY) is the most widely applied graft-versus-host disease (GVHD) prophylaxis regimen in T-cell replete haploidentical bone marrow transplantation (haplo-BMT). Although PT-CY has met with great success in the haplo-BMT arena by suppressing GVHD, patients without acute GVHD have high relapse rates. One strategy to reduce relapse rates being explored by others is a dosage reduction of PT-CY. We have taken a different approach in evaluating whether partially replacing PT-CY with post-transplantation bendamustine (PT-BEN) would be advantageous, an idea based on our preclinical research identifying several beneficial immunomodulatory properties of BEN. We therefore initiated and completed a Phase Ia trial to evaluate the progressive substitution of PT-CY with PT-BEN (ClinicalTrials.gov identifier NCT02996773). We compared outcomes between 13 patients with high-risk hematologic malignancies who received PT-CY/BEN and 31 contemporaneous haplo-BMT recipients treated with the same myeloablative conditioning regimens but receiving only PT-CY. We found that partial replacement of PT-CY with PT-BEN (PT-CY/BEN) on day +4 was well tolerated and associated with significantly earlier trilineage engraftment. We also report favorable trends toward significant improvements on univariate and multivariate analyses with PT-CY/BEN compared with PT-CY with respect to rates of chronic GVHD (hazard ratio [HR], .08; 95% confidence interval [CI], .005 to 1.11; $P = .06$), and GVHD-free relapse-free survival (GRFS) (HR, .22; 95% CI, .05 to .86; $P = .039$). Our human trial has now transitioned to Phase Ib, which will further evaluate the safety and potential benefits of PT-CY/BEN. Herein we also expand our pediatric, adolescent, and young adult experience to 31 patients, demonstrating overall survival, progression-free survival, and GRFS at 3 years of 85.6%, 76.1%, and 58.2%, respectively, in a largely racial/ethnic minority cohort. PT-CY/BEN appears to be a promising treatment option that requires further evaluation.

INTRODUCTION

We previously reported that in a preclinical murine haploidentical bone marrow transplantation (haplo-BMT) model, replacement of post-transplantation cyclophosphamide (PT-CY) with post-transplantation bendamustine (PT-BEN) is protective against late graft-versus-host disease (GVHD) [1]. We also have documented distinct immunomodulatory properties of BEN in several mismatched murine BMT models [1-6]. Compared with CY, BEN given pretransplantation or post-transplantation led to significant changes in the proportion, phenotype, and function of multiple immune subsets, including myeloid-derived suppressive cells (MDSCs) [2] and

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dendritic cell (DC) [3,5] subsets, yielding tolerant T cells with a striking absence of GVHD but preservation of the T cell-dependent graft-versus-leukemia (GVL) effect [4]. We therefore initiated a Phase Ia trial (ClinicalTrials.gov identifier NCT02996773) to evaluate the progressive substitution of PT-CY with PT-BEN to determine the maximum tolerated dose. Our interim analysis of the Phase Ia data showed that partial replacement of PT-CY with PT-BEN was associated with early trilineage engraftment, lower incidence of acute GVHD (aGVHD) and chronic GVHD (cGVHD), and reduced cytomegalovirus (CMV) reactivation [7]. Here we report our clinical findings from the completed Phase Ia and compare them with data from contemporaneous haplo-BMT recipients conditioned with the same regimens but receiving only PT-CY. To verify the safety and potential advantages of this platform, we have now transitioned to Phase Ib, using PT-CY on day +3 with PT-BEN on day +4 (PT-CY/BEN).

METHODS

Patients

Patients with hematologic malignancies who underwent T-cell replete haplo-BMT after myeloablative conditioning (MAC) between October 2015 and June 2021, were included in this study. Eligible patients were age 0 to 44 years who had no matched related donor, met the organ criteria allowing for MAC, and had no evidence of active untreated infection. Haplo-BMT were performed at the pediatric (n = 31) and adult (n = 13) hematopoietic cell therapy and transplantation (HCTT) services at Banner University Medical Center, Tucson, Arizona.

Transplantation Procedure

According to their diagnosis and disease characteristics, patients received either fractionated total body irradiation (TBI) followed by fludarabine (FLU) or a busulfan (BU), FLU, and melphalan (MEL) combination (Table 1) [8]. The first 3 patients receiving TBI-FLU were conditioned with fractionated TBI of 333 cGy given once daily for 3 days, whereas the later 20 patients received 200 cGy twice daily (1200 cGy total dose with lungs shielded to 900 cGy by custom cerrobend blocking) on days -8, -7, and -6, followed by FLU 30 mg/m^2 on days -5, -4, -3, and -2 [8,9]. Twenty-one patients received BU at .8 mg/kg i.v. every 6 hours for a total of 12 doses (days -8 to -6), targeting an average area under the curve of 4.1 to 4.5 mg·h/L every 6 hours. BU was followed by FLU 30 mg/m^2 on days -5, -4, -3, and -2, which was increased to 40 mg/m^2 in the later 6 patients, and MEL 100 mg/m^2 on day -2 [8,10]. All patients received bone marrow grafts on day 0.

GVHD Prophylaxis

Thirty-one patients received PT-CY 50 mg/kg on days +3 and +4, and the other 13 patients were treated as part of our Phase Ia/Ib single-institution trial of PT-CY/BEN. This Phase Ia/Ib trial was approved by our Institutional Review Board, and all patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki. Phase Ia was a standard 3+3 dose-escalation design, with the first 3 cohorts receiving PT-CY (mg/kg)/PT-BEN (mg/m^2) 40/20, 20/60, and 0/90 on day +4. All patients received PT-CY 50 mg/kg on day +3. Cohort 4 patients received PT-CY/BEN 40/20 on day +3 and only PT-BEN 90 mg/m^2 on day +4. Cohort 3 dosing (day +3, CY 50 mg/kg; day +4, BEN 90 mg/m^2) was deemed the maximum tolerated dose as we transitioned to Phase Ib with patient 13, who received PT-CY on day +3 and PT-BEN on day +4. In addition, all 44 patients received mycophenolate mofetil on days +5 through +28 and tacrolimus starting on day +5, with a target level of 5 to 8 ng/mL. In the absence of GVHD, tacrolimus was tapered starting on day +70 to +90 and discontinued by day +120 to +180. GVHD was graded according to the consensus criteria for grading aGVHD and cGVHD [11,12].

Supportive Care

Antifungal prophylaxis primarily with voriconazole was administered to all patients. Patients received i.v. pentamidine for *Pneumocystis jirovecii* prophylaxis and acyclovir for herpes simplex and varicella virus prophylaxis. Only 2 PT-CY patients received letermovir prophylaxis. Biweekly polymerase chain reaction (PCR) monitoring for CMV and weekly PCR for adenovirus, Epstein-Barr virus (EBV), and human herpesvirus 6 (HHV-6) were performed until discharge from the hospital and then at least every other week during the first 100 days post-transplantation. All patients underwent transplantation in positive-pressure HEPA-filtered rooms on HEPA-filtered units.

Table 1
Patient, Disease, and Transplantation Characteristics

Characteristic	PT-CY/BEN (N = 13)	PT-CY (N = 31)	*P* Value
Age, yr, median (range)	21.4 (9-42)	20.5 (.6-44)	.80
Male sex, n (%)	8 (62)	21 (68)	.74
Race/ethnicity, n (%)			.52
African American	1 (8)	3 (10)	
Native American	0 (0)	1 (3)	
Asian	0 (0)	1 (3)	
White Hispanic	6 (46)	15 (48)	
White non-Hispanic	6 (46)	11 (35)	
Diagnosis, n (%)			.72
ALL	6 (46)	16 (52)	
AML/MDS	3 (23)	10 (32)	
AUL/MLL	1 (8)	1 (3)	
CML	1 (8)	2 (6)	
NHL	2 (15)	1 (3)	
HD	0 (0)	1 (3)	
Clinical service, n (%)			.72
Pediatric	10 (77)	21 (68)	
Adult	3 (23)	10 (32)	
Pretransplantation status, n (%)			.40
CR1	5 (38)	12 (39)	
CR2	3 (23)	11 (35)	
>CR2	3 (23)	4 (13)	
Other	2 (15)	4 (13)	
Pretransplantation status, n (%)			.30
Prior HCT	0 (0)	4 (13)	
Prior CAR-T cell therapy	1 (8)	3 (10)	
Disease Risk Index, n (%)			.99
Low	1 (8)	3 (10)	
Intermediate	9 (69)	20 (65)	
High	3 (23)	7 (23)	
Very high	0 (0)	1 (3)	
Lansky/Karnofsky Performance Status, n (%)			
90-100	9 (69)	19 (61)	.74
≤80	4 (31)	12 (39)	
HCT Comorbidity Index, n (%)			.20
≤2	10 (77)	17 (55)	
≥3	3 (23)	14 (45)	
Conditioning, n (%)			.99
TBI-FLU	7 (54)	16 (52)	
BU-FLU-MEL	6 (46)	15 (48)	
Donor age, yr, median (range)	27 (15-57)	35.5 (16-62)	.40
Sex mismatch, n (%)			.74
Female→male	3 (23)	9 (29)	
Male→female	1 (8)	4 (13)	
None	9 (69)	18 (58)	
HLA match, n (%)			.16
5/10	11 (84)	18 (58)	
6/10	1 (8)	9 (29)	
7/10	1 (8)	2 (6)	
8/10	0 (0)	1 (6)	
9/10	0 (0)	1 (6)	
RBC incompatibility, n (%)			.45
Major	4 (31)	6 (19)	

(*continued*)

Table 1 (*Continued*)

Characteristic	PT-CY/BEN (N = 13)	PT-CY (N = 31)	*P* Value
Minor	2 (15)	4 (13)	
None	7 (54)	21 (68)	

MDS indicates myelodysplastic syndrome; AUL/MLL, acute undifferentiated or mixed lineage leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma; HD, Hodgkin disease; CR, complete remission; CAR, chimeric antigen receptor.

Donor Selection

Donors were first-degree relatives determined to be HLA-haploidentical based on high-resolution typing at HLA-A, -B, -Cw, -DRB1, and -DQB1. None of the patients had anti-donor HLA antibodies. Major and minor ABO incompatibilities necessitating donor RBC reduction using Hespan (6% hetastarch in .9% sodium chloride injection) for RBC sedimentation or plasma reduction are noted in Table 1.

Engraftment and Donor Chimerism Monitoring

Granulocyte colony-stimulating factor (G-CSF) was started on day +5 at 5 μg/kg/day and continued until an absolute neutrophil count (ANC) of 2.5×10^9/L was achieved for 3 consecutive days. The day of myeloid engraftment was defined as the first of 3 consecutive days with an ANC of $\geq .5 \times 10^9$/L. The day of platelet engraftment was considered the first of 3 consecutive days with a platelet count $\geq 20 \times 10^9$/L without receipt of a platelet transfusion in the previous 7 days. Donor chimerism was evaluated on days +28, +100, +180, and +365 by short tandem repeats in peripheral blood or bone marrow.

Statistical Analysis

Comparisons of patient characteristics and outcome variables between the PT-CY and PT-CY/BEN groups were performed using the Fisher exact test for categorical variables and the Mann-Whitney/Wilcoxon rank-sum test for continuous variables. Time-to-event endpoints were estimated using cumulative incidence curves and Kaplan-Meier curves, with comparisons done using log-rank tests. The associations between the clinical endpoints and patient characteristics were assessed using a Cox proportional hazards model for both univariate and multivariate analyses. Multivariate analysis first assessed the significance of PT-CY versus PT-CY/BEN adjusted for each covariate individually. Subsequent multivariate analysis was based on the covariates, including age, race/ethnicity, clinical service, conditioning regimen, diagnosis, remission status, Disease Risk Index, Hematopoietic Cell Transplantation Comorbidity Index, Lansky/Karnofsky Performance Status, donor-recipient sex mismatch, and donor age. For both univariate and multivariate analyses, the hazard ratio (HR), score test *P*value, and 95% confidence interval (CI) based on the Wald statistic are presented. Cox proportional hazards models for aGVHD II-IV, aGVHD III-IV, cGVHD, and severe cGVHD also were adjusted for competing risks due to disease relapse, second transplantation, or death.

RESULTS

Patient, Disease, and Transplantation Characteristics

The study cohort comprised 44 patients (66% males) with a median age of 21 years (range, .6 to 44 years) at the time of transplantation. Thirteen patients received a PT-CY/BEN combination, and 31 received PT-CY alone. Patient and transplantation characteristics are summarized in Table 1. Twelve of these patients were part of a 3+3 BEN dose escalation with CY deescalation Phase Ia trial (Table 2). The 13th patient is part of our Phase Ib trial, being conducted to further evaluate the safety and potential benefits of PT-CY/BEN compared with PT-CY alone. Baseline characteristics, including age, sex, race/ethnicity, diagnosis, clinical service, pretransplantation remission status, Disease Risk Index, Performance Status, HCT Comorbidity Index, conditioning regimen, donor age, sex mismatch, and HLA match were comparable in the PT-CY/BEN and PT-CY groups (Table 1). Ethnic and/or racial minorities constituted 61% of all patients, the majority of whom were Hispanic (48%). Acute lymphoblastic leukemia (ALL) was the most frequent diagnosis (50%), followed by acute myelogenous leukemia (AML)/myelodysplastic syndrome (30%). Conditioning was almost evenly split, with 52% of patients receiving TBI-FLU and 48% BU-FLU-MEL (Table 1). Univariate analysis of association of outcomes (relapse, nonrelapse mortality [NRM], OS, PFS, and GRFS) with patient, disease, and transplantation characteristics showed a significant increase in time to relapse for patients age >21 years (HR, 4.69; 95% CI, 1.01 to 21.73; $P = .03$) and those who underwent haplo-BMT on the adult clinical service (HR, 3.11; 95% CI, .95 to 10.24; $P = .048$), shortened PFS in patients who underwent haplo-BMT on the adult service (HR, 2.70; 95% CI, 1.01 to 7.22; $P = .04$) and improved GRFS in cases with a donor-recipient sex match (HR, .42; 95% CI, .18 to .99; $P = .04$) (Table 3).

Table 2
PT-CY/BEN: GVHD, Complications, Infections

Parameter	Phase Ia				Phase Ib
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	
Day +3 CY/BEN*	50/-	50/-	50/-	40/20	50/-
Day +4 CY/BEN	40/20	20/60	-/90	-/90	-/90
No. of patients	3	3	3	3	1
Graft failure					
Primary	-	-	-	-	-
Secondary	-	-	-	1	-
aGVHD					
None	1	1	2	1	-
Grade I	-	1	1	-	-
Grade II	2	1	-	1	-
Grade III	-	-	-	-	1
Grade IV	-	-	-	-	-
cGVHD					
None	3	3	2	2	1
Mild	-	-		-	-
Moderate	-	-	1	-	-
Severe	-	-	-	-	-
Bacteremia					
Gram+	1	2	2	-	-
Gram-	-	-	-	-	-
Fungal	-	-	-	-	-
CMV serostatus					
R-D+	-	-	-	-	-
R+D+	0/1	1/2	0/1	1/1	-
R+D-	0/1	0/1	0/2	1/1	1/1
BK viruria	2	-	-	1	-
VOD/SOS	-	-	-	-	-
TA-TMA	-	-	-	-	-
Dialysis	-	-	-	-	-
ICU admission	-	-	-	-	-
CRS	-	-	-	1	-
Relapse	1	1	-	1	-
Cause of death					
Relapse	1	1	-	-	-

* CY, mg/kg, BEN, mg/m^2.R indicates recipient; D, donor; VOD, veno-occlusive disease; SOS, sinusoidal obstruction syndrome, TA-TMA, transplantation-associated thrombotic microangiopathy.

Engraftment and Chimerism

The median cell dose was 4.05×10^6/kg CD34$^+$ cells in the PT-CY/BEN group, compared with 3.45×10^6/kg in the PT-CY group ($P = .298$) (Figure 1A). Patients treated with PT-CY/BEN had earlier trilineage engraftment, with a median time to an

Table 3
Univariate Analysis of Association of Outcomes with Patient, Disease, and Transplantation Characteristics

Characteristic	n	Relapse, HR (95% CI)	*P* Value	NRM, HR (95% CI)	*P* Value	OS, HR (95% CI)	*P* Value	PFS, HR (95% CI)	*P* Value	GRFS, HR (95% CI)	*P* Value
Age											
<21 yr	22	1	**.03**	1	.98	1	.17	1	.10	1	.21
>21 yr	22	4.69 (1.01- 21.71)		1.02 (.21-5.06)		2.47 (.65-9.34)		2.36 (.82-6.81)		1.74 (.72-4.21)	
Race											
Hispanic	21	1	.55	1	.51	1	.99	1	.94	1	.80
White	17	.66 (.17-2.58)		1.71 (.34-8.58)		.99 (.29-3.42)		1.04 (.36-2.92)		.89 (.36- 2.18)	
Diagnosis											
ALL	22	1	.26	1	.06	1	.12	1	.80	1	.55
AML	15	2.3 (.51-10.38)		0 (0-0)		.22 (.03-1.77)		.86 (.26-2.86)		.74 (.27-2.00)	
Clinic service											
Pediatric	31	1	**.048**	1	.78	1	.28	1	**.04**	1	.10
Adult	13	3.11 (.95-1.24)		1.27 (.23-6.99)		1.89 (.57-6.22)		2.70 (1.01-7.22)		2.05 (.86-4.86)	
Pretransplantation status											
CR1	17	1	.78	1	.99	1	.87	1	.92	1	.94
CR2	14	.77 (.17-3.46)		.93 (.13-6.74)		1.31 (.29-5.90)		.84 (.25-2.77)		.88 (.31-2.56)	
>CR2 or other	13	1.3 (.32-5.23)		1.05 (.15-7.52)		1.49 (.33-6.71)		1.07 (.33-3.52)		1.07 (.39-2.96)	
Disease Risk Index											
Low/Intermediate	33	1	.22	1	.35	1	.16	1	.28	1	.40
High-Very High	11	2.13 (.62-7.34)		2.19 (.39-12.23)		2.38 (.69-8.23)		1.78 (.61-5.18)		1.47 (.59-3.65)	
Lansky/Karnofsky Performance Status											
90-100	28	1	.22	1	.79	1	.91	1	.53	1	.72
≤89	16	.48 (.15-1.58)		1.26 (.23-6.87)		1.07 (.31-3.67)		.73 (.27-1.96)		.85 (.35-2.06)	
HCT Comorbidity Index											
≤2	27	1	.24	1	.77	1	.45	1	.14	1	.13
≥3	17	2.01 (.61-6.59)		1.27 (.25-6.35)		1.57 (.48-5.16)		2.07 (.77-5.56)		1.92 (.82-4.55)	
Conditioning											
TBI-FLU	23	1	.99	1	.86	1	.98	1	.88	1	.11
BU-FLU-MEL	21	1.02 (.31- 3.36)		1.15 (.23-5.70)		1.01 (.31-3.32)		.93 (.34-2.50)		.49 (.20-1.22)	
Donor age											
<40 yr	29	1	.59	1	.53	1	.28	1	.33	1	.51
>40 yr	15	1.38 (.42-4.52)		1.66 (.33-8.26)		1.9 (.58-6.25)		1.62 (.61-4.34)		1.34 (.55-3.24)	
Sex mismatch											
Yes	17	1	.60	1	.11	1	.21	1	.29	1	**.04**
No	27	.73 (.22-2.4)		.27 (.05-1.51)		.47 (.15-1.56)		.59 (.22-1.58)		.42 (.18-.99)	
Sex mismatch (F→M)											
Yes	12	1	.67	1	.31	1	.56	1	.87	1	.10
No	27	1.41 (.28-7.01)		.37 (.05-2.68)		.65 (.15-2.74)		.9 (.27-3.01)		.45 (.17-1.19)	

Significant *P* values are in bold type.

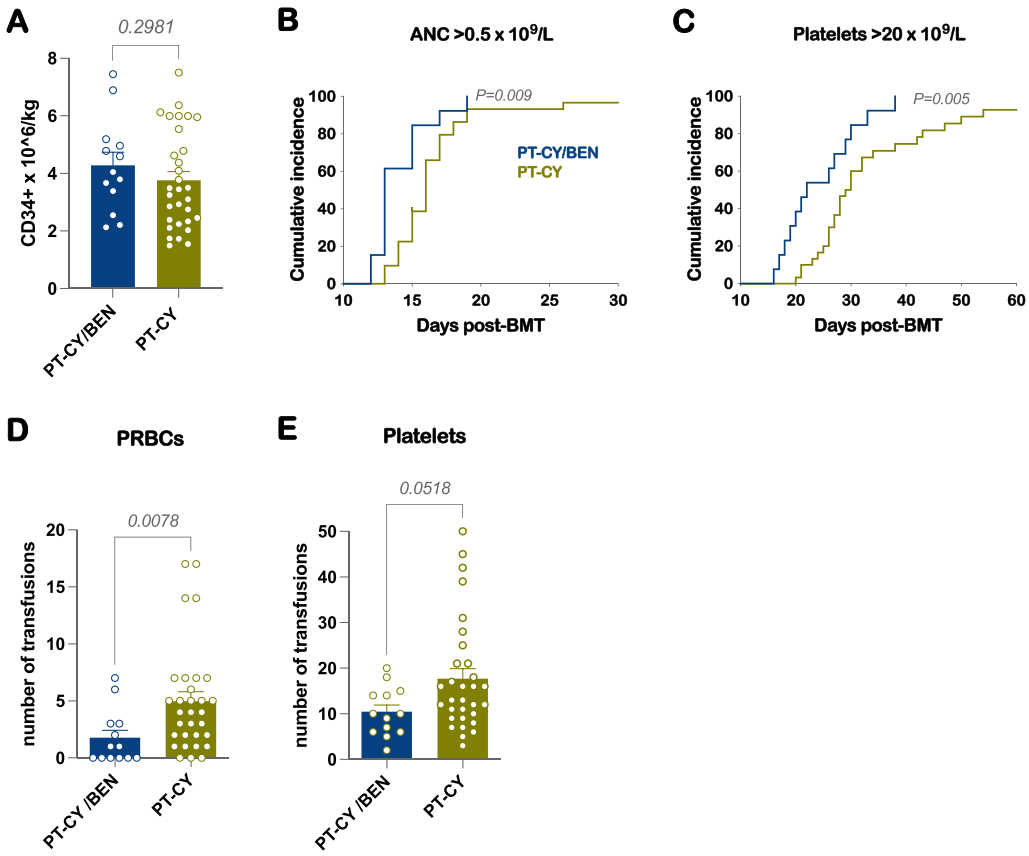

Figure 1. (A) Number of $CD34^+$ cells $\times$ 10^6/kg infused. (B) Time to an ANC of .5 $\times$ 10^9/L. (C) Time to a platelet count of 20 $\times$ 10^9/L. (D) Units of packed RBCs (PRBCs) transfused. (E) Units of platelets transfused.

ANC of .5 $\times$ 10^9/L of 13 days, compared with 16 days in those receiving PT-CY (P = .009) (Figure 1B). The PT-CY/BEN group also demonstrated earlier platelet engraftment, at a median of 22 days, compared with 29.5 days in the PT-CY group (P = .005) (Figure 1C). Consequently, the PT-CY/BEN patients required fewer RBC (P = .52) and platelet transfusions (P = .008) (Figure 1D,E). Trilineage engraftment was seen in 95.5% of patients, with all demonstrating complete donor chimerism in bone marrow on day +28 and in peripheral blood studies on days +100, +180, and +365.

Two patients developed graft failure (4.5%; one primary and the other secondary) following early CMV reactivation and ganciclovir treatment. The first patient had received PT-CY following infusion of 4.8 $\times$ 10^6 $CD34^+$ cells/kg and the second PT-CY/BEN (cohort 4; 40/20 on day +3 and 0/90 on day +4) (Table 2) and 2.2 $\times$ 10^6 $CD34^+$ cells/kg. Both patients were male with 6/10 HLA antigen-matched maternal donors, and both received BU-FLU-MEL (with 30 mg/m^2 of FLU). They were successfully salvaged after a second haplo-BMT (30 days and 46 days later) using a single-day conditioning regimen [13]. The PT-CY-treated patient died at 42 months after his first haplo-BMT of multiorgan failure, and the PT-CY/BEN-treated patient was alive and well at >20 months after his initial transplantation.

GVHD

The cumulative incidence of grade II-IV and grade III-IV aGVHD was 41.3% and 8.3%, respectively, in the PT-CY/BEN group and 46.2% and 20.8%, respectively, in the PT-CY alone (P = .52 and .29, respectively) (Figure 2A,B). The cumulative incidence of cGVHD was significantly lower at 9.1% following PT-CY/BEN, with 0% of patients developing severe cGVHD, compared with 44.1% and 20%, respectively, in the PT-CY group (P = .049 and .12) (Figure 2C,D). In multivariate analyses, PT-CY/BEN was not significantly associated with reduced grade II-IV aGVHD (HR, .71; 95% CI, .24 to 2.10; P = .53) and grade III-IV aGVHD (HR, .28; 95% CI, .03 to 2.71; P = .27), but with a trend toward less cGVHD overall (HR, .08; 95% CI, .005 to 1.11; P = .06) (Table 4).

NRM, Relapse, and Survival

The median duration of follow-up was 32 months (range, 8 to 55 months) in patients treated with PT-CY/BEN and 32 months (range, 13 to 74 months) in those receiving PT-CY. NRM was absent in patients receiving PT-CY/BEN; however, 25% of those treated with PT-CY died (P = .12), including 2 from bacterial sepsis, 2 from complications of cGVHD, and 1 secondary to multiorgan failure (Figure 2E). Relapse rates were comparable, with 26.6% in the PT-CY/BEN group and 31.1% in the PT-CY group (Figure 2F). The overall survival (OS) at 1 year and 3 years was similar, at 100% and 77.1%, respectively, for PT-CY/BEN and at 90.3% and 74.2%, respectively, for PT-CY (P = .38) (Figure 2G). Progression-free survival (PFS) also was comparable, at 83.9% at 1 year and 73.4% at 3 years with PT-CY/BEN versus 80.6% and 56.9%, respectively, with PT-CY (P = .25) (Figure 2H). Finally, we observed a trend toward improved GRFS with PT-CY/BEN at 75% at 1 year and 65.6% at 3 years versus 53.6% and 40.4%, respectively, with PT-CY (P = .11) (Figure 2I). In multivariate analysis, GRFS was significantly improved (HR, .22; 95% CI, .05 to .86; P = .039) in patients

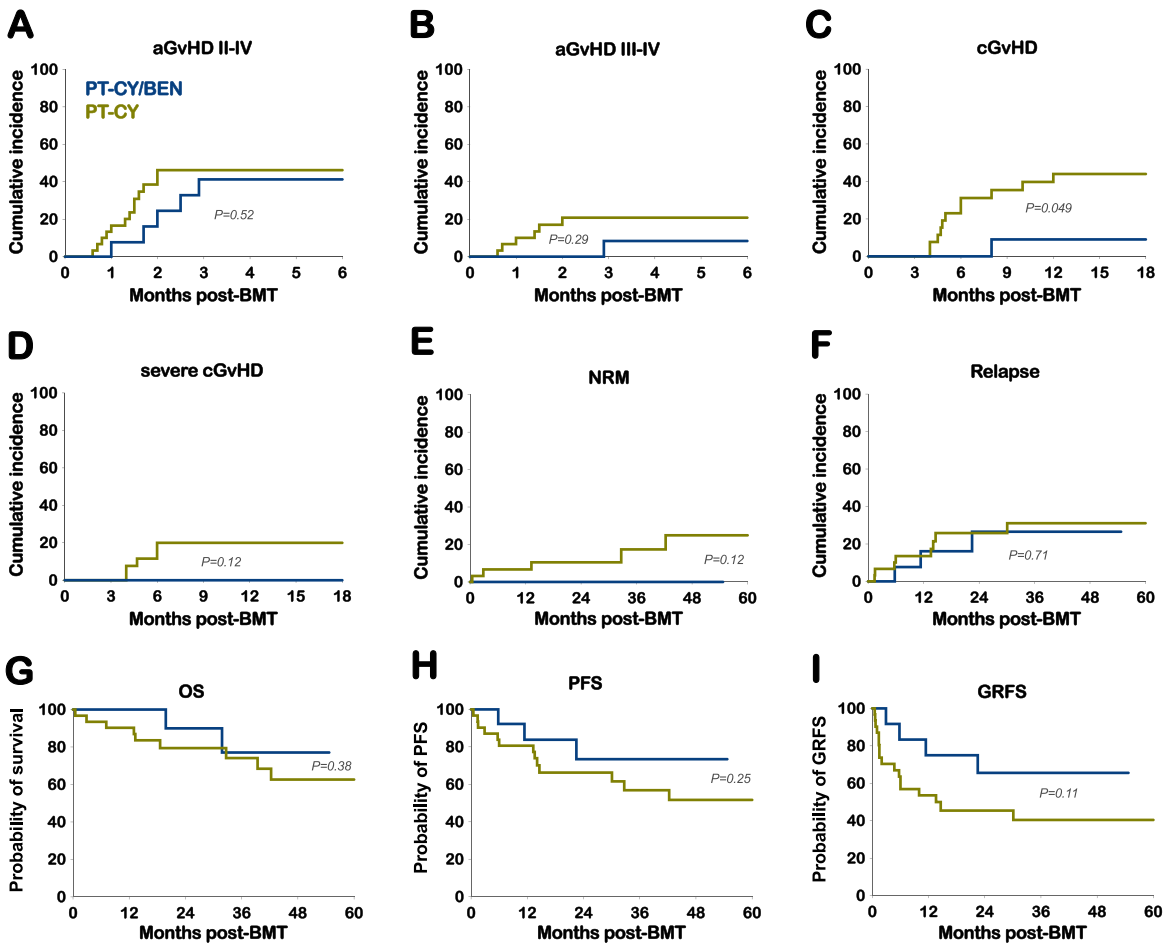

Figure 2. (A) Cumulative incidence of grade II-IV aGVHD. (B) Cumulative incidence of grade III-IV aGVHD. (C) Cumulative incidence of cGVHD. (D) Cumulative incidence of severe cGVHD. (E) Cumulative incidence of NRM. (F) Cumulative incidence of relapse. (G) Probability of OS. (H) Probability of PFS. (I) Probability of GRFS.

treated with PT-CY/BEN compared to PT-CY alone (Table 4). Similar to the univariate analysis, multivariate analysis did not reveal any significant improvement in OS and PFS for patients receiving PT-CY/BEN (HR, .25; 95% CI, .02 to 2.98 [P = .27] and HR, .30; 95% CI, .06 to 1.39 [P = .12], respectively) (Table 4).

Infections

CMV reactivation was frequently seen, with a median time to peak viral load of 40 days post-haplo-BMT (range, 8 to 53 days). The cumulative incidence was 36.4% in at-risk patients (ie, seropositive recipients and/or seropositive donors) receiving PT-CY/BEN, compared with 57.7% of at-risk PT-CY patients (P = .31) (Figure 3).

BK viruria was detected in 7 PT-CY patients (22.6%) with $>5 \times 10^8$ viral copies/mL and symptoms of BK hemorrhagic cystitis, compared with 3 PT-CY/BEN patients (23.1%) (P = .97) (Table 2). None of the patients in either group had clinically significant reactivation of EBV, HHV-6, or adenovirus warranting therapeutic intervention.

There was no significant difference in the incidence of gram-positive bacteremias between the PT-CY and PT-CY/BEN groups in the first 2 months after haplo-BMT, with 29% and 38.5%, respectively (P = .54), developing at least 1 positive culture (Table 2). Interestingly, no gram-negative bacteremias were observed in PT-CY/BEN patients, whereas 12.9% of the PT-CY patients had positive blood cultures, all for *Klebsiella pneumoniae* (P = .20). Similarly, no fungal infections occurred in PT-CY/BEN patients, whereas 1 PT-CY patient developed *Candida kruseii* fungemia.

Analysis of Patients Who Underwent Haplo-BMT on the Pediatric HCTT Service

Thirty-one pediatric, adolescent, and young adult patients age .6 to 27.2 years (median, 16.8 years) underwent their haplo-BMT on the pediatric HCTT service. Ten of these patients (32.2%) received PT-CY/BEN. NRM at 3 years was 6.5%, and the cumulative incidence of relapse was 6.9% at 1 year and 18.6% at 3 years (Figure 4A). The incidence of aGVHD grade II-IV and grade III-IV at 6 months was 43.6% and 14.4%, respectively (Figure 4B), whereas that of overall and severe cGVHD at 1 year was 23.3% and 7.7%, respectively (Figure 4C). OS, PFS, and GRFS at 3 years were 85.6%, 76.1%, and 58.2%, respectively (Figure 4D).

DISCUSSION

T cell-replete haplo-BMT with PT-CY has rapidly become an established alternative to unrelated HCT [14–16]. PT-CY following the Johns Hopkins reduced-intensity conditioning regimen has been associated with low rates of severe aGVHD and cGVHD [17,18]; however, approximately one-half of patients without aGVHD ultimately relapse [19]. This has prompted the use of MAC regimens [9,20-22], especially in younger patients, to reduce disease recurrence, but at a potential cost of increasing GVHD and NRM. Another strategy being explored to lessen

Table 4
Univariate and Multivariate Analysis of Outcomes

Outcome	Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Grade II-IV aGVHD				
PT-CY	1		1	
PT-CY/BEN	.71 (.25-2.01)	.52	.71 (.24-2.10)	.53
Grade III-IV aGVHD				
PT-CY	1		1	
PT-CY/BEN	.34 (.04-2.81)	.29	.28 (.03-2.71)	.27
cGVHD				
PT-CY	1		1	
PT-CY/BEN	.16 (.02-1.27)	**.049**	.08 (.005-1.11)	.06
Severe cGVHD				
PT-CY	1		1	
PT-CY/BEN	.00 (.00-.00)	.12	.00 (.00-.00)	.99
NRM				
PT-CY	1		1	
PT-CY/BEN	.00 (.00-.00)	.12	.00 (.00-.00)	.99
Relapse				
PT-CY	1		1	
PT-CY/BEN	.78 (.21-2.93)	.71	1.15 (.13-10.03)	.89
OS				
PT-CY	1		1	
PT-CY/BEN	.51 (.11-2.37)	.38	.25 (.02-2.98)	.27
PFS				
PT-CY	1		1	
PT-CY/BEN	.49 (.13-1.71)	.25	.30 (.06-1.39)	.12
GRFS				
PT-CY	1		1	
PT-CY/BEN	.42 (.14-1.25)	.11	.22 (.05-.86)	**.039**

Significant *P* values are in bold type.

relapse rates is PT-CY dosage reduction. Preclinical studies in mismatched murine BMT models reported by Kanakry et al. [23] have demonstrated benefits of a reduced PT-CY dose. This led to a clinical trial by the same investigator at the National Institutes of Health (ClinicalTrials.gov identifier NCT03983850) evaluating deescalation of PT-CY to one-half the dose of the current standard (25 mg/kg on days +3 and +4). Presentation of their early findings indicated that the reduced PT-CY dose maintained protection against severe aGVHD while promoting more rapid engraftment and also decreased early

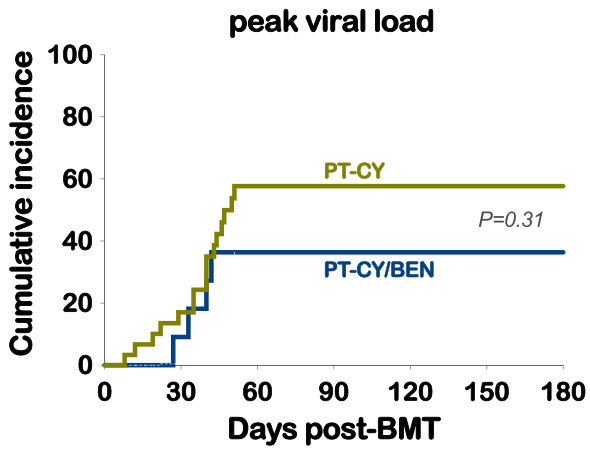

Figure 3. Cumulative incidence of CMV viremia and time to peak viral load.

post-transplantation toxicity. Others also have reported on the feasibility of reducing the PT-CY dose [24,25].

We have taken a different approach in evaluating whether partially replacing PT-CY with PT-BEN would be advantageous. Our preclinical research in mice supports the replacement of CY with BEN by demonstrating that PT-BEN was equally effective in preventing early GVHD and protecting against late GVHD while providing superior GVL compared with PT-CY [1]. BEN is a multipurpose agent that shows promise as a chemotherapeutic for a variety of cancers, as a conditioning regimen component for HCT, and as a lymphodepleting agent given before chimeric antigen receptor T cell therapy or donor leukocyte infusion [26-31]. Since our initial publication and using various preclinical models, our laboratory has delineated several immunomodulatory properties of BEN, elucidating its role in modulating GVHD and GVL [1-6]. Irrespective of whether BEN is given pretransplantation or post-transplantation, we have reliably observed decreased GVHD, increased GVL, and significant changes in the proportion and phenotype of multiple immune cell types [6]. These have included effects of BEN on MDSCs and DC subsets [2,3] and generation of tolerant T cells with a striking absence of GVHD, while preserving T cell-dependent GVL [4]. In addition, our in vitro studies have revealed that BEN increases the suppressive functions of MDSCs, skews DC generation toward cDC1s, promotes DC Flt3 expression, increases B cell production of IL-10, inhibits STAT3 phosphorylation, and suppresses B cell and T cell proliferation [1-6].

This work provided the foundation for further exploring the potential of PT-BEN in a first-in-human Phase Ia clinical trial. Phase Ia (ClinicalTrials.gov identifier NCT02996773) was a 3+3 dose escalation trial that accrued pediatric and young adult patients with hematologic malignancies. Our interim analysis included the first 3 cohorts receiving PT-CY 50 mg/kg on day +3 and on day +4 PT-CY (mg/kg)/PT-BEN (mg/m^2): 40/20, 20/60, and 0/90 (Table 2) [7]. PT-CY/BEN was well tolerated, with no dose-limiting toxicities. Since the publication of our interim analysis, we have completed enrollment of cohort 4. The first patient in cohort 4 experienced secondary graft failure following early CMV reactivation. The third patient in the same cohort developed cytokine release syndrome (CRS) and responded to a single dose of tocilizumab. CRS after PT-BEN was recently reported to occur in 70% of patients with advanced primarily refractory hematologic malignancies, who received much higher doses of PT-BEN (total dose, 140 to 280 mg/m^2) [32]. Of significance, however, is that 44% of the patients in that study did not receive tacrolimus or mycophenolate mofetil, and 85% received peripheral blood stem cell (PBSC) grafts rather than bone marrow grafts. CRS was observed in 1 of our 3 patients who received a dose of PT-BEN totaling 110 mg/m^2 but not in any patients in cohorts 1 to 3 (PT-BEN 20 to 90 mg/m^2) suggesting a possible increased risk of CRS with escalation of PT-BEN. Based on these 2 events, the prior dose level used in cohort 3 (day +3, CY 50 mg/kg; day +4, BEN 90 mg/m^2) was deemed the maximum tolerated dose as we transitioned to Phase Ib to further evaluate the safety and potential benefits of PT-CY/BEN compared to PT-CY alone and provide sample size estimates for the design of a potential Phase II multisite randomized trial. Our Phase Ia trial accrued patients up age 45 years. Two standard of care MAC regimens at our institution were used in all patients. TBI-FLU is generally used for ALL, and BU-FLU-MEL is used for myeloid malignancies and other diagnoses [7,8,33]. To include a comparable group in the present study, we analyzed all

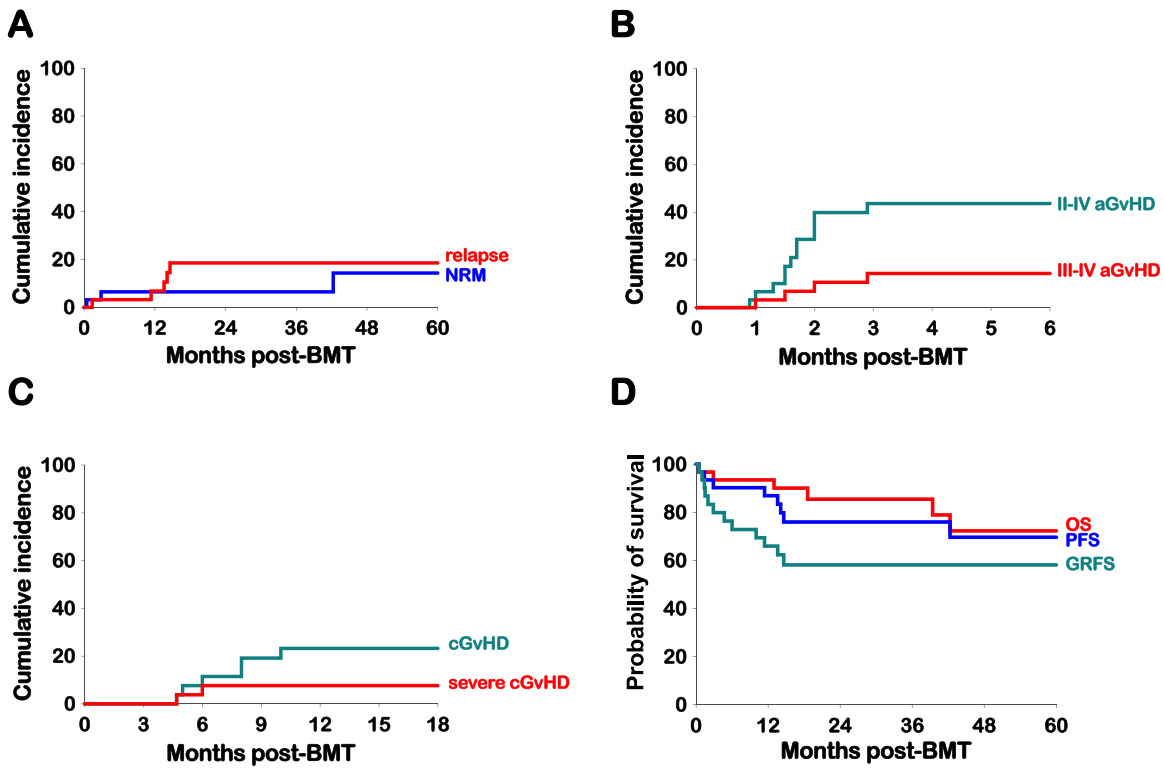

Figure 4. Outcomes of pediatric, adolescent, and young adult patients undergoing haplo-BMT on the pediatric service. (A) Cumulative incidence of NRM and relapse. (B) Cumulative incidence of grade II-IV and grade III-IV acute GVHD. (C) Cumulative incidence of cGVHD and severe cGVHD. (D) Probability of OS, PFS, and GRFS.

patients age <45 years undergoing T cell-replete haplo-BMT with PT-CY and the same 2 MAC regimens (n = 31). PT-CY has been considered safe against hematopoietic stem cells, because these cells express high levels of aldehyde dehydrogenase, thereby detoxifying CY [34,35]. However, BEN has multiple activities, as it contains a mechlorethamine group, a butyric acid side chain, and a benzimidazole ring. The alkylating properties provided by the mechlorethamine group are similar to CY, whereas the butyric acid increases water solubility and the benzimidazole ring is believed to function as a purine analog, affording antimetabolic characteristics [36-38]. Consequently, we were cautious in designing the Phase Ia trial, affording only gradual progression in the deescalation of CY and escalation of BEN starting at day +4 while leaving the dose of CY at day +3 unchanged. Despite our small numbers, we observed progressively improved trilineage engraftment and decreased transfusion requirements with escalation of PT-BEN and deescalation of PT-CY (Figure 1). Moreover, we are seeing favorable trends toward significant improvements in cGVHD and GRFS with PT-CY/BEN compared with PT-CY in univariate and multivariate analyses (Figure 2C,D,I, Table 4). We should note, however, that the cumulative incidence of cGVHD was higher in our PT-CY patients compared with that reported in studies using reduced-intensity conditioning and even MAC regimens [9,18,39,40].

Viral infections contribute to substantial transplantation-related morbidity and mortality in patients undergoing haplo-BMT, with CMV the leading culprit [41-44]. Our previous report of a reduced incidence of CMV reactivation in our interim analysis did not hold true with the additional PT-CY/BEN patients enrolled in the study [7]. Although CMV viremia was still less frequent following PT-CY/BEN, this difference was no longer significant (Figure 3). Two of the 31 patients receiving PT-CY were on letermovir (including 1 patient who reactivated CMV despite it), whereas none of the patients treated with PT-CY/BEN received CMV prophylaxis, because it was not part of the study protocol at that time. All patients responded to antiviral therapy primarily with ganciclovir or valganciclovir; however, as noted above, CMV contributed to secondary graft failure in a PT-CY/BEN patient.

As BK hemorrhagic cystitis is a recognized complication of high-dose CY therapy, one would expect an increased incidence of BK viruria compared with that seen in PT-CY/BEN patients. Although we did not observe a significant difference between the groups, it was notable that 2 of the 3 PT-CY/BEN patients developed clinically evident BK viruria were from cohort 1 (with the highest PT-CY doses of 50 mg/kg on day +3 and 40 mg/kg on day +4) (Table 2). Our results corroborate that BK hemorrhagic cystitis is a significant complication of PT-CY, reported to occur in one-third to one-half of patients undergoing haplo-BMT with PT-CY [45]. If PT-BEN emerges as a safe alternative to PT-CY, it may have the added advantage of the reduced hemorrhagic cystitis and renal complications associated with BK viremia. Regarding other viruses that may complicate transplantation, no patients in either group had clinically significant EBV, adenovirus, or HHV-6 reactivation. Ongoing immune reconstitution analyses from this trial may shed more light on potential differences in viral control.

Another potential drawback of high-dose PT-CY is cardiotoxicity. A recent study found a higher incidence of cardiac events (eg, left ventricular systolic dysfunction, arrhythmias, pulmonary edema, arrhythmias) occurring within the first 100 days post-transplantation in patients receiving PT-CY [46]. Not unexpectedly, the younger patients in our study did not show evidence of clinically significant cardiac adverse effects of PT-CY. The addition of PT-BEN was well tolerated without any major toxicities, such

as sinusoidal obstruction syndrome and thrombotic microangiopathy, and no admissions to the intensive care unit.

We recently reported on our haplo-BMT experience in 21 pediatric and young adult patients, and the present report expands this number to 31, which to date is the largest study from a single pediatric center in North America. The 10 additional mostly high-risk patients include 2 infants and a 2-year-old with *KMT2A*$^{+}$ AML, a patient with therapy-associated AML following treatment of Ewing sarcoma, 2 patients with *FLT3+* AML, a patient with ALL who had relapsed following matched sibling HCT and chimeric antigen receptor T cell therapy, and 3 patients with ALL in second or later complete remission. Three patients received PT-CY 40 mg/kg and PT-BEN 20 mg/m^2 on day +3 and PT-BEN 90 mg/m^2 on day +4 (Phase Ia, cohort 4), and 1 patient received only PT-CY 50 mg/kg on day +3 and only PT-BEN 90 mg/m^2 on day +4 (Phase Ib). The remaining 6 patients received PT-CY on both days. The present study expanded the median duration of follow-up to 32 months from the 25 months on the previous study.

Our patients' characteristics are comparable to those in a recent report of 29 pediatric and young adult patients undergoing haplo-BMT following MAC at Johns Hopkins [47]. Engraftment was similar in the 2 studies, as was time to a platelet count $\geq 20 \times 10^9$/L. However, in our cohort, the time to an ANC $\geq .5 \times 10^9$/L was considerably earlier—day +15 compared to day +24—even though our patients received a slightly lower CD34^{+} cell dose infusion (4.1×10^6/kg versus 5.4×10^6/kg). Thirty-two percent of our patients received PT-CY/BEN, which is associated with an earlier time to an ANC $\geq .5 \times 10^9$/L (Figure 1). We observed a higher incidence of aGVHD and an apparently slightly lower incidence of cGVHD compared with Symons et al. [47]. Finally, our 3-year OS and PFS were 85% and 76%, respectively, compared with 79% and 69% in the Johns Hopkins report, confirming that MAC followed by haplo-BMT and PT-CY is an effective approach for pediatric and AYA patients with high-risk hematologic malignancies.

In summary, our group has pioneered the use of PT-BEN, first in an experimental murine haplo-BMT model [1] and then by completion of a Phase Ia trial [7]. Although our clinical findings are preliminary, together with our published preclinical murine BMT studies, they provide evidence that partially substituting PT-CY with PT-BEN enhances engraftment and may favor survival with a trend toward reduced cGVHD and consequent improvement in GRFS. With the trial proceeding to Phase Ib, we hopefully can provide confirmation of the safety and efficacy of this post-transplantation prophylactic platform and its immunomodulatory effects.

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