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Development of Grade II Acute Graft-versus-Host Disease Is Associated with Improved Survival after Myeloablative HLA-Matched Bone Marrow Transplantation using Single-Agent Post-Transplant Cyclophosphamide

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A B S T R A C T

Post-transplant cyclophosphamide (PTCy) can be used as the sole immunosuppression after myeloablative conditioning (MAC) for HLA-matched bone marrow transplantation (BMT). However, the effects of graft-versus-host disease (GVHD) with this platform are undefined. We retrospectively analyzed 298 consecutive adult patients with hematologic malignancies who engrafted after MAC HLA-matched sibling donor (MSD; n = 187) or HLA-matched unrelated donor (MUD; n = 111) T-cell–replete BMT with PTCy 50 mg/kg on days +3 and +4. After MSD and MUD BMT, 35% and 57% of patients, respectively, developed grade II acute GVHD (aGVHD) by 100 days, 11% and 14% grade III to IV aGVHD by 100 days, and 9% and 16% chronic GVHD (cGVHD) by 1 year. In landmark analyses at 100 days after HLA-matched BMT, 4-year overall survival (OS) and progression-free survival (PFS) were 57% (95% confidence interval [CI], .49 to .67) and 40% (95% CI, .31 to .51) in patients without grades II to IV aGVHD, and 68% (95% CI, .59 to .78) and 54% (95% CI, .44 to .65) in patients with grade II aGVHD. In adjusted time-dependent multivariable analyses, grade II aGVHD was associated with improved OS (hazard ratio, .58; 95% CI, .37 to .89; $P = .01$) and PFS (hazard ratio, .50; 95% CI, .34 to .74; $P < .001$) after HLA-matched BMT with PTCy. The ability of PTCy to limit grades III to IV aGVHD and cGVHD while maintaining grade II aGVHD may contribute to its effectiveness, and further attempts to reduce aGVHD may be detrimental.

INTRODUCTION

In 1990 Horowitz et al. [1] demonstrated that mild acute graft-versus-host disease (aGVHD) or chronic graft-versus-host disease (cGVHD) improved, whereas severe GVHD worsened leukemia-free survival after HLA-matched sibling donor (MSD) T-cell–replete bone marrow transplantation (BMT). When compared with patients without GVHD, patients who developed all grades of aGVHD had a reduction in relapse with a remarkable absence of relapse events in patients with the most severe GVHD. However, beneficial effects on relapse were outweighed by an increase in mortality in patients with severe GVHD.

Post-transplant cyclophosphamide (PTCy) was developed to reduce GVHD and improve engraftment after HLA-haploidentical (haplo) BMT [2–7]. After haplo BMT with PTCy severe aGVHD and cGVHD incidences are low and not affected by the degree of HLA disparity, whereas grade II aGVHD rates are similar to other transplant platforms [8,9]. Importantly, we recently reported that grade II aGVHD is associated with improved progression-free survival (PFS) after haplo BMT with PTCy [9].

The success of PTCy in reducing severe aGVHD and cGVHD after haplo BMT led to the extension of its use to HLA-matched transplantation [10]. We have previously published on the efficacy of PTCy as a single-agent for GVHD prophylaxis after myeloablative conditioning and HLA-matched BMT [10–13]. Increasing interest in PTCy as a single-agent for GVHD

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prophylaxis after BMT is reflected by a growing number of reports [10–14] and an ongoing Blood and Marrow Transplant Clinical Trials Network study (BMT CTN 1301). Given the PFS benefit with grade II aGVHD in haplo BMT with PTCy, here examined the effects of grade II aGVHD on PFS in this separate cohort of patients receiving HLA-matched BMT with PTCy as the sole GVHD prophylaxis.

METHODS

Patient and BMT Procedures

Institutional Review Board approval was granted for this retrospective review of 298 consecutive patients transplanted for malignant hematologic diseases from the initiation of this approach in 2004 through December 31, 2012. Fourteen patients who developed primary or secondary graft failure (5 after MSD and 9 after HLA-matched unrelated donor [MUD] BMT) were excluded from the analysis. Patients did not receive donor lymphocyte infusion in the absence of relapse. Patients were treated with myeloablative conditioning consisting of busulfan with either fludarabine [12] or Cy [12] as previously published followed by a T-cell–replete bone marrow graft with a goal dose of 4×10^8 nucleated cells/kg recipient ideal body weight on day 0 followed by PTCy at 50 mg/kg ideal body weight i.v. with mesna on days +3 and +4. No additional immunosuppression was given unless patients developed extracutaneous aGVHD, grade IV cutaneous aGVHD, moderate/severe cGVHD, or required it for other reasons such as engraftment syndrome [15].

Outcome Definitions

Overall survival (OS) was defined from the date of transplant to death from any cause. PFS was defined as survival in the absence of relapse, progression, or unplanned treatment of disease persistence. Nonrelapse mortality (NRM) was defined as death in the absence of relapse, progression, or unplanned treatment of disease persistence. Patients who did not experience the outcome events were censored at the date of last follow-up. When estimating cumulative incidence, NRM was a competing risk for relapse and vice versa. When estimating the cumulative incidence of GVHD, competing risks were relapse, donor lymphocyte infusion, and death.

To diagnose and grade aGVHD and cGVHD, we used the modified Keystone [16] and the 2005 National Institutes of Health consensus criteria [17], respectively. GVHD scoring was performed by the first or second author, followed by an independent assessment by the Johns Hopkins GVHD specialist (J.B.-M.). Any disagreement in GVHD diagnosis or grading was discussed between the authors and a consensus reached. Patients with suspected maximal grade I aGVHD were included in the group of patients categorized as not having GVHD, unless they progressed to a higher grade of GVHD, at which time they were categorized in the corresponding higher grade GVHD group. The effect of grade I aGVHD on outcomes was not examined because, even in the presence of a biopsy, it can be difficult to distinguish mild aGVHD from other etiologies for early post-transplant rashes such as viral infection and drug allergy. Therefore, we did not believe that distinguishing grade I aGVHD from other post-transplant rashes could be determined with high accuracy and these patients were excluded.

Statistical Analysis

Data were locked on October 31, 2017. OS and PFS were estimated by the Kaplan-Meier method [18]. The cumulative incidence of relapse and NRM were determined by Gray's method [19]. The point estimators of OS, PFS, relapse, and NRM were reported via landmark analysis using the landmark time points of 100 days and 1 year for grade II aGVHD and cGVHD, respectively. Because of the small number of grade III to IV events in the landmark datasets, the effect of grades III to IV aGVHD were not conducted in landmark analysis, but only examined in time-dependent multivariable models. The landmark time points were chosen based on prior literature [1,20–22] and a time point that allowed us to capture sufficient number of patients who developed a GVHD event. In our data all grade II aGVHD events occurred within 100 days after BMT, and 90% of cGVHD events occurred within 1 year after BMT.

In exploratory analyses the landmark results were similar in patients undergoing MSD and MUD BMT with PTCy (Supplemental Figures 1 to 4); therefore, the cohorts were combined to increase power and for simplicity of presentation. The associations of GVHD with subsequent outcomes were evaluated in multivariable models that included all study patients and treated the status of GVHD as a time-dependent covariate using Cox proportional hazard models for OS and PFS and cause-specific hazard models for relapse and NRM.

The final models for multivariable analyses were based on backward elimination with $P < .10$ as inclusion criterion where patient age and GVHD status were always retained in the models. In addition, when we did not force patient age and GVHD status in selection criteria, but generated the model using only backward elimination with $P < .10$ as inclusion criterion, the GVHD status variable still satisfied unforced inclusion criteria for all our interested outcomes (OS, PFS, relapse, and NRM) as did age for OS, PFS, and NRM. Thus, the GVHD status would have been retained in the model even without forcing criteria.

Covariates at the beginning of the multivariable model included donor (MUD versus MSD), diagnosis (acute leukemia including acute myeloid leukemia and acute lymphoblastic leukemia, myelodysplastic syndrome, or myeloproliferative neoplasms including chronic myelomonocytic leukemia and chronic myeloid leukemia, lymphoma, and multiple myeloma), pretransplant disease status (complete remission without minimal residual disease versus minimal residual disease/active disease) [23–27], disease risk index (DRI; low or intermediate versus high/very high) [28], hematopoietic cell transplantation–specific comorbidity index (HCT-CI) [29], cytomegalovirus serostatus of recipient and donor (recipient negative–donor negative, recipient positive–donor negative, recipient negative–donor positive, and recipient positive–donor positive), donor age (as a continuous variable), female-into-male allografting, nucleated cell graft dose (as a continuous variable and as a separate model by low, intermediate, or high dose levels), $CD34^+$ cell graft dose (as a continuous variable and as a separate model by low, intermediate, or high dose levels), $CD3^+$ cell graft dose (as a continuous variable and as a separate model by low, intermediate, high), and year of BMT cut at the median (2004 to 2008 versus 2009 to 2012).

Analyses were carried out with the statistical software R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria). $P \leq .05$ was considered as statistically significance for hypothesis generating.

RESULTS

Patient and Transplant Characteristics

Patient and transplant characteristics are shown in Table 1. Supplemental Table 1 shows patient and transplant characteristics separately by MSD and MUD. Median follow-up times for

Table 1
Patient and Transplant Characteristics (N = 298)

Variables	Value
Female	144 (48%)
Median (range) age at BMT, yr	48 (18-66)
Transplant platforms	
MSD	187 (63%)
MUD	111 (37%)
Pretransplant status	
CR	125 (42%)
MRD/active disease	173 (58%)
DRI	
Low-risk	26 (9%)
Intermediate-risk	173 (58%)
High or very high-risk	99 (33%)
HCT-CI ≥ 3	113 (38%)
BMT year	
2004-2008	163 (55%)
2009-2012	135 (45%)
Patient–donor cytomegalovirus	
Patient negative, donor negative	96 (32%)
Patient positive, donor negative	78 (26%)
Patient negative, donor positive	51 (17%)
Patient positive, donor negative	72 (24%)
Unavailable	1
Median (range) donor age, yr	43 (17-76)
Female into male allografting, yes	60 (20%)
Median (range) nucleated cell dose x 10^8/kg recipient ideal body weight	4.14 (0.88-8.82)
Median (range) $CD3^+$ infusion x 10^7/kg recipient ideal body weight	3.82 (0.6,9.59)
Median (range) $CD34^+$ infusion x 10^6/kg recipient ideal body weight	3.69 (0.97,9.87)
Diagnosis	
Acute leukemia (AML or ALL)	185 (62%)
MDS or MPN (including CMML, CML)	58 (20%)
Lymphoma	46 (15%)
Multiple myeloma	9 (3%)

Values are n (%) unless otherwise defined. CR indicates complete remission; MRD, minimal residual disease; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; CMML, chronic myelomonocytic leukemia; CML, chronic myeloid leukemia.

all patients were 5.3 years (range, 17 days to 10.16 years) after MSD BMT and 4.4 years (range, 34 days to 10.57 years) after MUD BMT based on the reverse Kaplan-Meier method. Median patient ages were 50 years and 49 years at the time of MSD and MUD BMT, respectively. The most common diagnoses in both cohorts were acute leukemia comprising 57% and 71% for MSD and MUD BMT, respectively, followed by myelodysplastic syndrome/myeloproliferative neoplasms, consisting of 17% and 23%, respectively. The DRI for most patients was intermediate risk, with very few low risk patients in either cohort. HCT-CI scores were ≥3 in approximately 40% of both MSD and MUD recipients.

GVHD Incidence

After MSD BMT, the 100-day cumulative incidence of grade II aGVHD was 35% (95% CI, 28% to 42%), grades III to IV aGVHD was 11% (95% CI, 6% to 15%), and 1-year cumulative incidence of cGVHD was 9% (95% CI, 5% to 13%). The corresponding median onset for grade II aGVHD was 38 days (range, 17 to 94), grades III to IV aGVHD was 51 days (range, 20 to 136), and cGVHD was 132 days (range, 52 to 311). After MUD BMT, the 100-day cumulative incidence of grade II aGVHD was 57% (95% CI, 47% to 66%), grades III to IV aGVHD was 14% (95% CI, 8% to 21%), and 1-year cumulative incidence of cGVHD was 16% (95% CI, 9% to 23%). The corresponding median onset for grade II aGVHD was 33 days (range, 15 to 98), grades III to IV aGVHD was 46 days (range, 22 to 219), and cGVHD was 130 days (range, 29 to 682) (Table 2). Among 128 patients who experienced grade II aGVHD in either the MSD or MUD cohorts, 58% had skin-only involvement, whereas 42% of events involved either the liver or gastrointestinal tract with or without skin involvement. Thirty-one percent of patients who had grade II aGVHD with liver or gastrointestinal involvement transitioned to grades III to IV aGVHD, whereas 14% of patients with skin-only grade II aGVHD progressed to grades III to IV aGVHD.

OS at Landmark Time Points

After MSD BMT, the 100-day OS was 89% (95% CI, 84% to 93%) in the entire MSD cohort. The 100-day OS was 86% (95% CI, 80% to 93%) in patients without grades II to IV aGVHD by 100 days, 98% (95% CI, 95% to 100%) in patients who had developed maximal grade II aGVHD by 100 days, and 80% (95% CI, 64% to 100%) in patients who had developed grades III to IV aGVHD by 1 year. After MSD BMT the 1-year OS was 69% (95% CI, 62% to 76%) in the entire MSD cohort, 68% (95% CI, 60% to 78%) in patients without GVHD by 1 year, and 76% (95% CI, 59% to 100%) in patients who had developed cGVHD by 1 year.

Table 2
Summary of GVHD Characteristics by Patients with HLA-MSD and HLA-MUD BMT

	MSD (n = 187)	MUD (n = 111)
Grade II aGVHD		
Total number of events	65	63
Median onset after BMT, days (range)	38 (17-94)	33 (15-98)
100 days CuI (95% CI)	.35 (.28-.42)	.57 (.47-.66)
Grades III-IV aGVHD		
Total number of events	23	19
Median onset after BMT, days (range)	51 (20-136)	46 (22-219)
100 days CuI (95% CI)	.11 (.06-.15)	.14 (.08-.21)
cGVHD		
Total number of events	17	20
Median onset after BMT, days (range)	132 (52-311)	130 (29-682)
1 year CuI (95% CI)	.09 (.05-.13)	.16 (.09-.23)

CuI indicates cumulative incidence.

After MUD BMT, the 100-day OS was 89% (95% CI, 84% to 95%) in the entire MUD cohort. The 100-day OS was 88% (95% CI, 79% to 98%) in patients without grades II to IV aGVHD by 100 days, 94% (95% CI, 88% to 100%) in patients who had developed maximal grade II aGVHD by 100 days, and 75% (95% CI, 57% to 100%) in patients who had developed grades III to IV aGVHD by 100 days. After MUD BMT the 1-year OS was 73% (95% CI, 65% to 82%) in the entire cohort, 74% (95% CI, 61% to 89%) in patients without GVHD by 1 year, and 83% (95% CI, 67% to 100%) for patients who had developed cGVHD by 1 year.

Landmark Analyses at 100 Days for Grade II aGVHD

Outcomes of MSD and MUD were similar in exploratory landmark analysis (Supplemental Figures 1 to 4); therefore, the cohorts were combined (HLA-matched BMT with PTCy) for the landmark and multivariable analyses. In landmark analyses at 100 days after HLA-matched BMT, 4-year OS and PFS were 57% (95% CI, .49 to .67) and 40% (95% CI, .31 to .51), respectively, in patients without grades II to IV aGVHD, and 68% (95% CI, .59 to .78) and 54% (95% CI, .44 to .65) in patients with grade II aGVHD (Figure 1A,B). In landmark analysis at 100 days after HLA-matched BMT, the 4-year cumulative incidences of relapse and NRM were 49% (95% CI, .39 to .59) and 11% (95% CI, .05 to .17) in patients without grades II to IV aGVHD and 33% (95% CI, .24 to .43) and 13% (95% CI, .06 to .20) in patients with maximal grade II aGVHD (Figure 1C,D).

Landmark Analyses at 1 Year for cGVHD

In landmark analysis at 1 year after HLA-matched BMT, 4-year OS and PFS were 71% (95% CI, .62 to .81) and 57% (95% CI, .45 to .71) in patients without GVHD and 79% (95% CI, .63 to 1) and 77% (95% CI, .59 to 1) in patients with cGVHD (Figure 2A,B). In landmark analyses at 1 year after HLA-matched BMT, the 4-year cumulative incidences of relapse and NRM were 41% (95% CI, .28 to .54) and 2% (95% CI: 0 to .05) in patients without GVHD and 5% (95% CI: 0 to .16) and 18% (95% CI: 0 to .37) in patients with cGVHD (Figure 2C,D).

Multivariable Modeling for Survival Endpoints Using GVHD as a Time-Dependent Covariate

In recipients of HLA-matched BMT, when compared with patients without GVHD, patients who had experienced maximal grade II aGVHD had a significantly improved OS (hazard ratio [HR], .58; 95% CI, .37 to .89; $P = .01$) and PFS (HR, .50; 95% CI, .34 to .74; $P < .001$) (Table 2). When compared with patients without GVHD, patients who had experienced grades III to IV aGVHD had significantly worse OS (HR, 1.78; 95% CI, 1.09 to 2.92; $P = .02$) and no significant difference in PFS (HR, 1.29; 95% CI, .82 to 2.05; $P = .27$). Finally, when compared with patients without GVHD, patients who had experienced cGVHD had no significant difference in OS (HR, 0.65; 95% CI, .34 to 1.22; $P = .18$) but significantly improved PFS (HR, .48; 95% CI, .26 to .87; $P = .02$) (Table 2). High or very high-risk disease by DRI (HR, 2.03 [95% CI, 1.43 to 2.88] and 1.83 [95% CI, 1.27 to 2.63] for OS and PFS, respectively) and higher HCT-CI scores as a continuous variable (HR, 1.14 [95% CI, 1.05 to 1.25] and 1.07 [95% CI, .99 to 1.16] for OS and PFS, respectively) were associated with worse OS and PFS. If DRI was removed from the model, then pretransplant disease status retained significance for decreased OS in patients with minimal residual disease positivity or active disease when compared with complete remission without minimal residual disease. Intermediate nucleated cell graft doses (3.71 to 4.53×10^8/kg recipient ideal body weight) improved OS and PFS when compared with lower doses ($<3.71 \times 10^8$/kg recipient ideal body weight). We

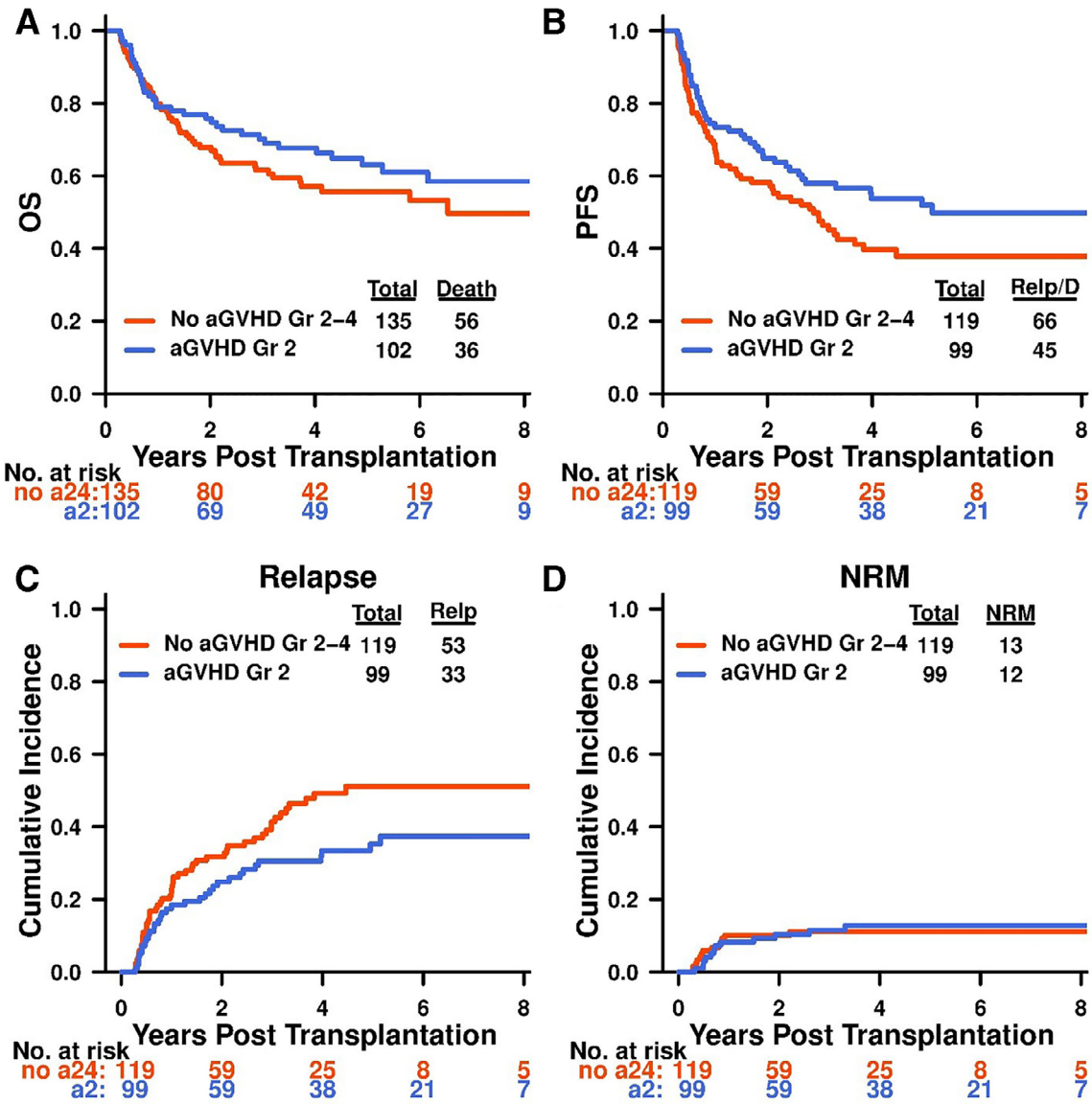

Figure 1. Landmark analyses: effects of grade II aGVHD on survival outcomes. *Curves were truncated at 8 years after BMT. (A) In patients who were alive at day 100 after HLA-matched BMT with PTCy, the probabilities of OS were compared. OS was higher in patients who had developed maximal grade II aGVHD (aGVHD Gr 2; *dark blue line*) when compared with patients who had not developed grades II to IV aGVHD (No aGVHD Gr 2-4; *orange line*). (B) In patients who were alive and who had not relapsed at day 100 after HLA-matched BMT with PTCy, the probabilities of PFS were compared. PFS was higher in patients who had developed maximal grade II aGVHD (aGVHD Gr 2*; dark blue line*) when compared with patients who had not developed grades II to IV aGVHD (No aGVHD Gr 2-4; *orange line*). (C) In patients who were alive and had not relapsed at day 100 after HLA-matched BMT with PTCy, the cumulative incidences of relapse were compared. Relapse was less frequent in patients who had developed maximal grade II aGVHD (aGVHD Gr 2*; dark blue line*) when compared with patients who had not developed grades II to IV aGVHD (No aGVHD Gr 2-4; *orange line*). (D) In patients who were alive at day 100 after HLA-matched BMT with PTCy, the cumulative incidences of NRM were compared. NRM was no different in patients who had developed maximal grade II aGVHD (aGVHD Gr 2; *dark blue line*) when compared with patients who had not developed grades II to IV aGVHD (No aGVHD Gr 2-4; *orange line*). No aGVHD Gr 2-4 indicates patients without grades II to IV aGVHD; aGVHD Gr 2, patients with grade II aGVHD; No., number; Relp, relapse.

performed an additional multivariable model for OS separating outcomes for grade II aGVHD by organ involvement (Supplemental Table 2). Patients who developed skin-only grade II aGVHD had significantly better OS when compared with patients without grades II to IV aGVHD (HR, .48; 95% CI, .28 to .84; $P = .01$). Patients who had grade II aGVHD of the liver or gastrointestinal tract had no statistically significant difference in OS when compared with patients without grades II to IV aGVHD (HR, .75; 95% CI, .41 to 1.36; $P = .34$). Other variables in the model (patient age, DRI, HCT-CI, and graft cell dose) retained similar effects.

In recipients of HLA-matched BMT, when compared with patients without GVHD, those who developed maximal grade II aGVHD had a significantly lower relapse rate (cause-specific HR [CSHR], .48; 95% CI, .31 to .75; $P = .001$) and no significant difference in NRM (CSHR, .58; 95% CI, .25 to 1.38; $P = .22$) (Table 3). When compared with patients without GVHD, those with grades III to IV aGVHD had no significant difference in relapse (CSHR, .53; 95% CI, .25 to 1.11; $P = .09$) and significantly higher rate of NRM (CSHR, 4.63; 95% CI, 2.32 to 9.25; $P < .001$). Finally, when compared with patients without GVHD, those with cGVHD had a significantly lower relapse rate (CSHR, .25;

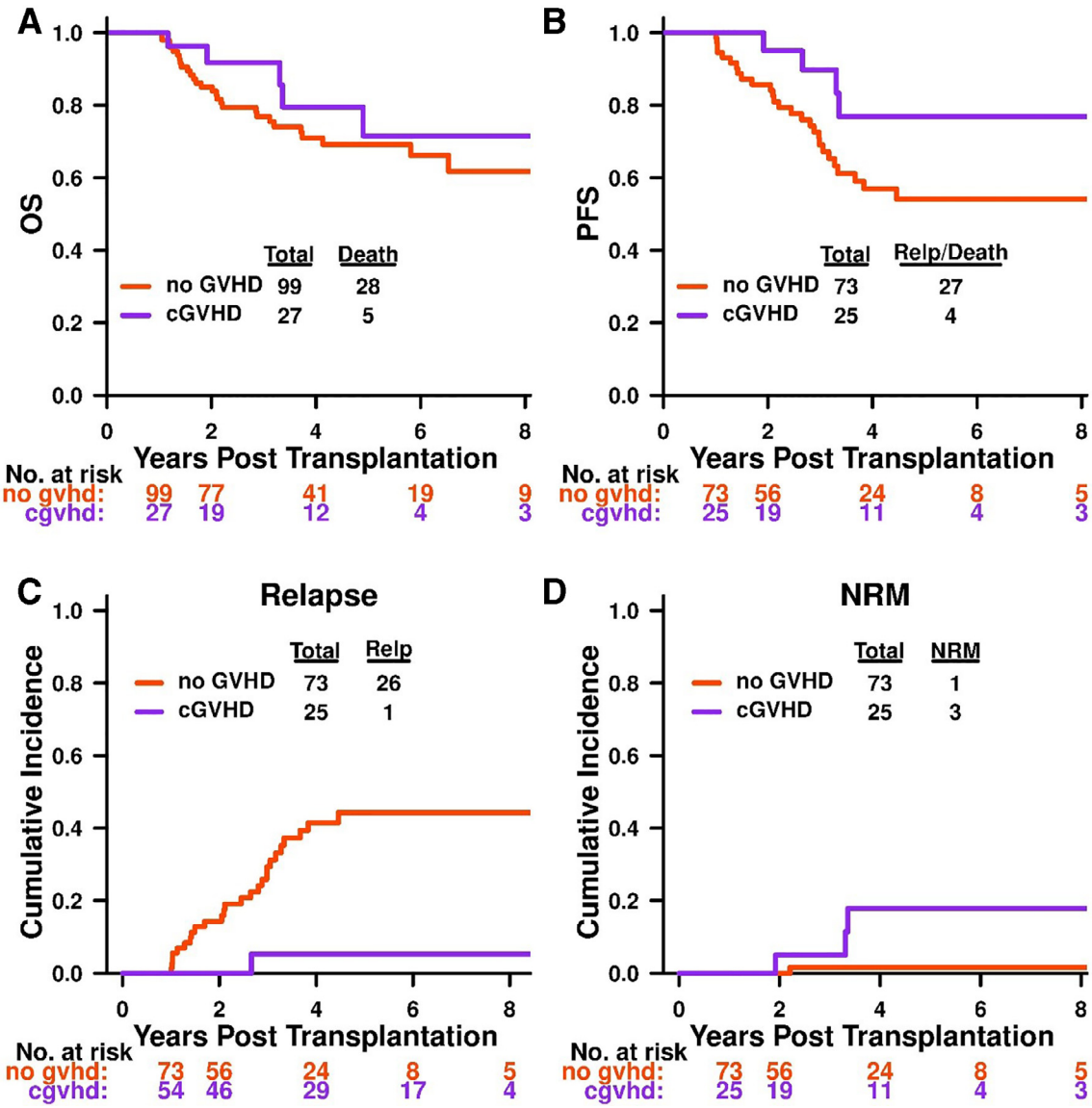

Figure 2. Landmark analyses: effects of cGVHD on survival endpoints. *Curves were truncated at 8 years after BMT. (A) In patients who were alive at 1 year after HLA-matched BMT with PTCy, the probabilities of OS were compared. OS for patients who had developed cGVHD (cGVHD; *purple line*) was similar when compared with patients who had not developed GVHD (no GVHD; *orange line*). (B) In patients who were alive and who had not relapsed at 1 year after HLA-matched BMT with PTCy, the probabilities of PFS were compared. PFS was higher in patients who had developed cGVHD (cGVHD; *purple line*) when compared with patients who had not developed GVHD (no GVHD; *orange line*). (C) In patients who were alive and had not relapsed at 1 year after HLA-matched BMT with PTCy, the cumulative incidences of relapse were compared. Relapse was lower in patients who had developed cGVHD (cGVHD; *purple line*) when compared with patients who had not developed GVHD (no GVHD; *orange line*). (D) In patients who were alive at 1 year after HLA-matched related BMT with PTCy, the cumulative incidences of NRM were compared. NRM was higher in patients who had developed cGVHD (cGVHD; *purple line*) when compared with patients who had not developed GVHD (no GVHD; *orange line*).

95% CI, .11 to .58; $P = .001$) and a tendency toward higher NRM (CSHR, 2.27; 95% CI, .89 to 5.76; $P = .08$) (Table 4). High or very high-risk disease by DRI and higher HCT-CI scores as a continuous variable were associated with more relapse and NRM, respectively. Intermediate and high nucleated cell graft doses and female into male allografting were all significantly associated with less relapse, but were not retained in the NRM model. High $CD34^+$ cell graft dose was associated with less NRM.

DISCUSSION

Prior work in HLA-matched T-cell–replete BMT showed that mild GVHD was associated with improved survival, whereas moderate/severe GVHD worsened outcomes [1]. Unfortunately, no platforms to date have successfully dissociated aGVHD from cGVHD or promoted mild GVHD in the absence of an increase in severe GVHD. Current immunosuppressive strategies used to prevent GVHD concomitantly diminish the anti-cancer effects of donor T-cells, increasing the risk of relapse. However, limiting the duration of immunosuppression has been associated in many platforms with an increased risk of severe aGVHD and cGVHD. We have previously shown that high-dose PTCy is an effective single-agent prophylactic strategy after HLA-matched BMT [10–13] and seems to reduce severe aGVHD and cGVHD without concomitant decreases in the incidence of grade II aGVHD. Similar to recent studies in haplo BMT with PTCy [9] and umbilical cord transplantation [30] we demonstrate that grade II aGVHD is associated with significantly improved OS and PFS in HLA-matched BMT with PTCy. We believe that this is due to

Table 3
Multivariable Model for OS and PFS after HLA-Matched BMT with PTCy

Covariates	OS		PFS	
	HR (95% CI)	*P*	HR (95% CI)	*P*
GVHD status (t)				
No GVHD (t)	1		1	
Grade II aGVHD (t)	.58 (.37-.89)	.01	.50 (.34-.74)	<.001
Grades III-IV aGVHD (t)	1.78 (1.09-2.92)	.02	1.29 (.82-2.05)	.27
cGVHD (t)	.65 (.34-1.22)	.18	.48 (.26-.87)	.02
Patient age (continuous)	1.02 (1.00-1.03)	.05	1.01 (1-1.03)	.08
DRI				
Low or intermediate-risk	1		1	
High or very high-risk	2.03 (1.43-2.88)	<.001	1.83 (1.27-2.63)	.001
HCT-CI	1.14 (1.05-1.25)	.002	1.07 (.99-1.16)	.08
Pretransplant status	Not retained			
CR			1	
MRD/active disease			1.50 (1.03-2.19)	.04
Female into male allografting, yes vs no	Not retained		.64 (.42-.97)	.04
Nucleated cell graft dose				
Low $<3.71 \times 10^8$/kg	1		1	
Int 3.71-4.53 $\times 10^8$/kg	.42 (.26-.67)	<.001	.51 (0.34-.76)	.001
High $>4.53 \times 10^8$/kg	.95 (.61-1.45)	.79	.78 (.52-1.15)	.20
$CD34^+$ graft dose				
Low $<3.26 \times 10^6$/kg	1		1	
Int 3.26-4.65 $\times 10^6$/kg	.82 (.54-1.26)	.37	.75 (.52-1.10)	.15
High $>4.65 \times 10^6$/kg	.67 (.42-1.05)	.08	.68 (.45-1.02)	.06
BMT year				
2004-2008	1		1	
2009-2012	.74 (.51-1.06)	.10	.76 (.56-1.04)	.08

(t) indicates time-dependent variable; Int, intermediate.

retention of the graft-versus-tumor effect with grade II aGVHD, but absence of the long-term morbidity and late mortality associated with moderate and severe cGVHD. The replication of our findings in haplo BMT [9] in this HLA-matched cohort treated with PTCy suggests that PTCy successfully modulates alloreactivity; thus, most mild aGVHD cases do not progress to higher grades of aGVHD or to cGVHD nor increase NRM, yet anti-tumor efficacy is maintained.

Cy was one of the first agents shown to be effective in controlling GVHD in animal models [31,32]. Subsequent mouse studies demonstrated that tolerance to disparate minor histocompatibility antigens could be induced if high-dose Cy was

Table 4
Multivariable Model for Cumulative Incidence of Relapse and NRM after HLA-Matched BMT with PTCy

Covariates	Relapse		NRM	
	CSHR (95% CI)	*P*	CSHR (95% CI)	*p*
GVHD status (t)				
No GVHD(t)	1		1	
Grade II aGVHD (t)	.48 (.31-.75)	.001	.58 (.25-1.38)	.22
Grades III-IV aGVHD (t)	.53 (.25-1.11)	.09	4.63 (2.32-9.25)	<.001
cGVHD (t)	.25 (.11-.58)	.001	2.27 (.89-5.76)	.08
Patient age (continuous)	1.00 (.98-1.02)	.96	1.06 (1.03-1.09)	<.001
DRI				
Low or intermediate-risk	1		Not retained	
High or very high-risk	3.16 (2.18-4.59)	<.001		
HCT-CI	Not retained		1.16 (1.02-1.31)	.02
Pretransplant status				
CR	Not retained		Not retained	
MRD/active disease				
Female into male allografting, yes vs. no	.53 (.31-.91)	.02	Not retained	
Cytomegalovirus serostatus				
Pt neg, dn neg	Not retained		1	
Pt pos, dn neg			2.09 (.96-4.55)	.06
Pt neg, dn pos			1.37 (.52-3.57)	.52
Pt pos, dn pos			1.65 (.75-3.62)	.21
Nucleated cell graft dose				
Low $<3.71 \times 10^8$/kg	1		Not retained	
Int 3.71-4.53 $\times 10^8$/kg	.44 (.28-.70)	<.001		
High $>4.53 \times 10^8$/kg	.58 (.38-.91)	.02		
$CD34^+$ graft dose				
Low $<3.26 \times 10^6$/kg	Not retained		1	
Int 3.26-4.65 $\times 10^6$/kg			1.00 (0.53-1.88)	.99
High $>4.65 \times 10^6$/kg			.37 (0.16-.84)	.02
BMT year				
2004-2008	1		Not retained	
2009-2012	.70 (.48-1.02)	.06		

given early after antigen exposure [31–36] and that this could be accomplished while preserving the anti-leukemic effects of the graft [37]. We have now demonstrated, clinically, what was demonstrated years ago in murine experiments: that control of severe GVHD with preservation of anti-leukemic effects can be seen with PTCy given at specific dosing and timing after allografting. Furthermore, we have demonstrated similar effects with differing PTCy-based BMT platforms: HLA-matched BMT and haplo BMT [9].

This current study has several limitations. First, we did not explore the effects of grade I aGVHD because of the difficulty in distinguishing it from other common post-transplant rashes that may be multifactorial or related to post-transplant medications or viral infections (such as human herpesvirus-6). Second, our study only included patients transplanted between 2004 and 2012 and does not account for the increasing use of post-transplant interventions (such as tyrosine kinase inhibitors including sorafenib, midostaurin, nilotinib, dasatinib etc.), which may promote mild GVHD and further improve outcomes. Finally, fully comprehending the effect of cGVHD is difficult because its occurrence is rare with this platform. Although cGVHD decreased relapse in our study, this came at the cost of a tendency toward increase in NRM, which may have an even greater impact with longer follow-up and outweigh the benefit in relapse reduction.

Our work may also have broader implications beyond PTCy platforms. Given the improvements in survival associated with grade II aGVHD in this and prior studies [1,30,38], we propose that grade II aGVHD be distinguished from grades III to IV aGVHD as a clinical trial endpoint in studies of malignant disease to avoid cataloging “the good” with “the bad” [9]. We also note that the rate of progression from grade II aGVHD to grades III to IV aGVHD is low with PTCy platforms, particularly with skin-only grade II aGVHD. This supports the avoidance of systemic immunosuppression to treat skin-only grade II aGVHD, when possible, because most cases can be watched expectantly without necessitating the reinitiation of immunosuppression; such an approach could limit side effects, infectious risk, and maintain the graft-versus-tumor effects of GVHD. We have previously shown that PTCy minimizes the global immunosuppressive burden experienced by patients undergoing HLA-matched BMT [15], and this work emphasizes the importance of exploring the further minimization of pharmacologic agents post-transplant in other platforms such as reported recently by our group in haplo BMT with PTCy [39]. Although this and other recent studies [9,15,39,40] suggest that potent anti-tumor immune responses are maintained with PTCy, fully deciphering these interactions requires further mechanistic studies.

In summary, we propose that grade II be separated from grades III to IV aGVHD as a clinical trial endpoint and that systemic treatment of skin-only grade II aGVHD be used cautiously or even avoided to limit negation of beneficial anti-tumor effects. In addition, we propose that further efforts to reduce aGVHD with PTCy platforms may not be beneficial to patients with malignant diseases. Finally, the ability of PTCy to limit grades III to IV aGVHD while maintaining mild acute GVHD may contribute to its effectiveness.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.12.767.

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