REGULAR ARTICLE

blood advances

Posttransplant cyclophosphamide vs tacrolimus–based GVHD prophylaxis: lower incidence of relapse and chronic GVHD

Katie Maurer,[1] Vincent T. Ho,[1] Eno Inyang,[1] Corey Cutler,[1] John Koreth,[1] Roman M. Shapiro,[1] Mahasweta Gooptu,[1] Rizwan Romee,[1] Sarah Nikiforow,[1] Joseph H. Antin,[1] Catherine J. Wu,[1] Jerome Ritz,[1] Robert J. Soiffer,[1] and Haesook T. Kim[2]

[1]Department of Medical Oncology and [2]Department of Data Science, Dana-Farber Cancer Institute, Harvard School of Public Health, Boston, MA

Key Points

- Unrelated-donor PBSCT with PTCY was associated with a reduced relapse rate compared with tacrolimus-based prophylaxis (25% vs 34%; $P = .027$).
- Moderate-to-severe GVHD was reduced in the PTCY cohort compared with the tacrolimus cohort (12% vs 36%; $P < .0001$).

The ability of posttransplant cyclophosphamide (PTCY) to facilitate haploidentical transplantation has spurred interest in whether PTCY can improve clinical outcomes in patients with HLA-matched unrelated donors undergoing peripheral blood stem cell transplantation (PBSCT). We investigated our institutional experience using PTCY-based graft-versus-host disease (GVHD) prophylaxis compared with conventional tacrolimus-based regimens. We compared overall survival, progression-free survival (PFS), relapse, nonrelapse mortality, and acute and chronic GVHD in 107 adult patients receiving a PTCY-based regimen vs 463 patients receiving tacrolimus-based regimens for GVHD prophylaxis. The 2 cohorts were well balanced for baseline characteristics except that more patients in the PTCY cohort having received 7-of-8–matched PBSCT. There was no difference in acute GVHD. All-grade chronic GVHD and moderate-to-severe chronic GVHD were substantially reduced in patients receiving PTCY compared with in those receiving tacrolimus-based regimens (2-year moderate-to-severe chronic GVHD: 12% vs 36%; $P < .0001$). Recipients of PTCY-based regimens also had a lower incidence of relapse compared with recipients of tacrolimus-based regimens (25% vs 34% at 2-years; $P = .027$), primarily in patients who received reduced intensity conditioning. This led to improved PFS in the PTCY cohort (64% vs 54% at 2 years; $P = .02$). In multivariable analysis, the hazard ratio was 0.59 ($P = .015$) for PFS and the subdistribution hazard ratio was 0.27 ($P < .0001$) for moderate-to-severe chronic GVHD and 0.59 ($P = .015$) for relapse. Our results suggest that PTCY prophylaxis is associated with lower rates of relapse and chronic GVHD in patients who receive HLA-matched unrelated donor PBSCT.

Introduction

Cyclophosphamide (Cy) is a highly immunosuppressive antineoplastic drug that remains a mainstay of therapy in many hematologic malignancies.[1] In blood and marrow hematopoietic cell transplantation (HCT), Cy has been used for decades as part of conditioning regimens to prevent graft rejection by suppressing the host immune system. In the posttransplant setting, pioneering work

Submitted 19 January 2023; accepted 23 April 2023; prepublished online on *Blood Advances* First Edition 8 May 2023; final version published online 27 July 2023. https://doi.org/10.1182/bloodadvances.2023009791.

The data that support the findings of this study are available upon reasonable request from the corresponding author, Haesook T. Kim (htkimc@jimmy.harvard.edu) after publication of the article for one year.

The full-text version of this article contains a data supplement.

from Johns Hopkins University demonstrated that administration of high-dose posttransplantation Cy (PTCY) can inhibit both graft rejection and graft-versus-host disease (GVHD), making it possible to cross human leukocyte antigen (HLA) barriers and allow haploidentical stem cell transplantation (haplo-HCT).[2] Based on this finding, a series of clinical trials with PTCY in haplo-HCT were conducted and demonstrated similar outcomes to matched related donor (MRD) or unrelated donor (URD) transplantation with respect to survival, relapse, and GVHD.[3-5] These critical studies revolutionized donor selection for allogeneic HCT by enabling haplo-HCT with PTCY for patients who did not have HLA-matched donors.[3-5] Recent mechanistic studies revealed that PTCY prevents GVHD by limiting functional alloreactivity in donor T cells and favoring more rapid recovery of regulatory T cells, thereby inducing tolerance and abrogating inflammation.[6,7] Because other strategies for GVHD prevention reportedly increase the risk of relapse through loss of a graft-versus-tumor (GVT) effect, there has been concern that the reduction in GVHD with PTCY may be associated with higher relapse rates.[8] However, rates of relapse after haplo-HCT with PTCY do not appear to be higher compared with MRD or URD HCT.[9-11] Nevertheless, the role, and the exact setting, of PTCY in mitigating relapse risk is unknown.

Based on its success in haplo-HCT, there is great interest in assessing whether PTCY-based GVHD prophylaxis regimens can be expanded beyond haplo-HCT to further improve outcomes and possibly replace conventional calcineurin inhibitor (CNI)-based GVHD prophylaxis regimens for MRD or URD peripheral blood stem cell transplantation (PBSCT). Indeed, in recent years, the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) has conducted a series of randomized clinical trials to investigate the efficacy of PTCY compared with tacrolimus (TAC)-based prophylaxis in the nonhaploidentical transplantation setting.[12-14] In 2 of these trials (BMT CTN 1203, 1703), PTCY was associated with lower incidences of acute and chronic GVHD.[12,14] Before BMT CTN 1703, a phase 3 trial conducted by the Dutch group (HOVON-96) also reported a lower incidence of acute and chronic GVHD in the PTCY group.[15] Another phase 3 trial (BMT CTN 1301), which used PCTY alone without CNI after myeloablative conditioning bone marrow transplantation, showed a significantly decreased cumulative incidence of relapse (CIR) in the PTCY arm compared with the control arm (13.9% vs 25.6%; $P = .037$) with no difference in other outcomes.[13] All these studies, however, allowed various conditioning regimens, assuming that the effect of conditioning regimen on transplant outcome is independent of the effect of prophylactic regimen, despite the fact that these 2 regimens are administered within a short period of time. To complement these randomized clinical trials, we hereby report our center's experience with PTCY in nonhaploidentical transplantation to further explore the settings in which PTCY confers the greatest benefit. In this study, we compared transplant outcome between TAC-based and PTCY-based prophylaxis. We then investigated in which conditioning regimen setting PTCY exerts the most benefit with a particular focus on whether PTCY in combination with less-intensive conditioning regimens affects relapse rate and GVHD after URD PBSCT.

Methods

Patients

The Blood and Marrow Transplant Data Repository of the Dana-Farber Cancer Institute was queried to identify all patients aged ≥18 years who underwent allogeneic PBSCT from an URD between 1 January 2018 and 31 December 2021. In total, 606 consecutive transplants were identified from 570 distinct patients who received PBSCT for hematologic malignancies. For patients who underwent >1 allogeneic HCT, only the first was included in this study. Of these 570 patients, 463 (81%) patients received TAC-based GVHD prophylaxis, and 107 (19%) patients received PTCY-based prophylaxis. The TAC-based prophylaxis in this study included tacrolimus with methotrexate (TAC/MTX; n = 322), tacrolimus with methotrexate and rapamycin (sirolimus) (TAC/MTX/Rap; n = 116), and tacrolimus with rapamycin (TAC/Rap; n = 25). All PCTY-based prophylaxis (n = 107) was administered with tacrolimus and mycophenolate mofetil (PTCY/TAC/MMF). Most patients (85%) received 8 of 8 (A, B, C, DRB1) matched unrelated PBSCT. The study was approved by the institutional review board of the Dana-Farber/Harvard Cancer Center and conducted in accordance with the Declaration of Helsinki.

GVHD prophylaxis and conditioning regimen

PTCY-based GVHD prophylaxis consisted of administration of high-dose Cy (50 mg/kg) on day +3 and +4 after transplantation, along with TAC and MMF beginning on day +5. TAC was tapered at approximately day +100, and MMF was discontinued between day +28 and +35. The TAC/MTX regimen consisted of TAC beginning on day 3 before transplantation and MTX administered on days +1, +3, +6, and +11 after transplantation. TAC was tapered beginning from approximately day +100 if there was no evidence of active GVHD and discontinued from approximately day +180. For the TAC/Rap or TAC/MTX/Rap regimens, TAC and MTX were administered as described earlier, whereas Rap was started on day +3 and administered daily.[16] Rap (sirolimus) was tapered from approximately day +100 if there was no evidence of active GVHD, and discontinued at approximately day +180. In recent years, our center has transitioned to using PTCY/TAC/MMF primarily for HLA-mismatched transplants. Otherwise, no specific criteria were used to determine use of prophylactic regimen, and decision to use PTCY- vs TAC-based GVHD prophylaxis in the matched-transplant setting was at the discretion of the transplant physician.

Conditioning intensity was defined by standard criteria and reduced intensity conditioning (RIC) regimens in this study included fludarabine 120 mg/m^2 with intravenous busulfan at doses of 3.2 mg/kg (Flu/Bu1) or 6.4mg/kg (Flu/Bu2), fludarabine 120mg/m^2 with melphalan 100 mg/m^2 to 140 mg/m^2 (Flu/Mel), or fludarabine with Cy and low-dose total body irradiation (TBI) 200 cGy (Flu/Cy/TBI). Myeloablative conditioning (MAC) regimens included high-dose busulfan (12.8 mg/kg) with fludarabine (Flu/Bu4), Cy 3600 mg/m^2 with TBI of 1200 cGy (Cy/TBI), fludarabine 90 mg/m^2 with TBI of 1200 cGy (Flu/TBI), and fludarabine 120 mg/m^2 with melphalan 140 mg/m^2 and busulfan 9.6 mg/kg (Flu/Bu/Mel).

Study end points and statistical analysis

End points in this study included progression-free survival (PFS), overall survival (OS), nonrelapse mortality (NRM), relapse, acute

GVHD, chronic GVHD, the composite end point GVHD-free and relapse-free survival (GRFS), neutrophil and platelet engraftment, and cytomegalovirus (CMV) reactivation with the primary interest in relapse. The Kaplan-Meier method was used to estimate PFS, OS, and GRFS whereas cumulative incidence of NRM, relapse, GVHD and CMV reactivation were estimated in the context of a competing risks framework. The log-rank test and Gray test were used for group comparisons. To assess the effect of GVHD prophylaxis on time-to-events in the presence of other risk factors, multivariable Cox regression analysis for OS, PFS, and GRFS and Fine and Gray regression analysis for cumulative incidence of NRM, relapse, and chronic GVHD were performed. Definitions of end points and detailed statistical analysis are provided in the supplemental Materials.

Results

Patients

The baseline characteristics are summarized in Table 1. Both cohorts were well balanced with respect to age, sex, donor sex, Karnofsky performance score (KPS), disease, CMV serologic status, HCT comorbidity index (HCT-CI), disease risk index at transplant, and conditioning intensity. Because of our current practice, patients who received PTCY-based prophylaxis were more likely to receive 7 of 8 matched PBSCT (53.3%) compared with the TAC group (5.8%) ($P < .0001$). The proportion with donor age ≥40 years was also somewhat higher (15.9% vs 9.1%; $P = .051$) in the PTCY group.

Clinical outcomes

For the entire cohort, the median follow-up among survivors was 24.3 months (range, 8.6-57). Two-year PFS was 64% in the PTCY group and 54% in the TAC group ($P = .02$) (Figure 1A; supplemental Table 1). Two-year OS was 66% in the PTCY group and 62% in the TAC group ($P = .067$) (Figure 1B; supplemental Table 1). Cumulative incidence of NRM in the PTCY and TAC groups was similar (11% vs 12% at 2 years, respectively; $P = .73$) (supplemental Table 1). However, the CIR was lower in the PTCY group compared with the TAC group (25% vs 34% at 2 years; $P = .027$). (Figure 1D; supplemental Table 1)

Six-month cumulative incidence of grade 2-4 acute GVHD was 20% in both groups ($P = .64$) and grade 3-4 acute GVHD was 7.5% and 8.6% ($P = .53$) in the PTCY and TAC group, respectively. (Figure 2A-B; supplemental Table 1). Consistent with prior reports, the 2-year cumulative incidence of chronic GVHD was 32% and 58% ($P = .00008$) and moderate-to-severe chronic GVHD was 12% and 36% ($P < .00001$) in the PTCY and the TAC group, respectively (Figure 2C-D; supplemental Table 1). Because of the lower CIR and moderate-to-severe chronic GVHD, the composite end point GRFS was substantially higher in the PTCY group compared with that in the TAC group (2-year GRFS, 56% vs 30%; $P = .00001$; Figure 1C). These outcomes are summarized and presented as a stack plot in Figure 3A. To further assess the impact of PTCY on outcomes, we performed multivariable regression analysis using a Cox model for OS, PFS, and GRFS and the Fine and Gray competing risks regression model for NRM, relapse, and moderate-to-severe chronic GVHD. Consistent with the results from the univariable analysis, subdistribution hazard ratio (sHR) comparing the PTCY-based with TAC-based prophylaxis was 0.51 ($P = .009$) for relapse and 0.27 for moderate-to-severe chronic GVHD ($P < .0001$). HR was 0.44 for GRFS ($P < .0001$) for GRFS, 0.59 for PFS ($P = .015$) and 0.63 for OS ($P = .05$). There was no difference in acute GVHD and NRM (Figure 3B). In these multivariable models, baseline characteristics such as age, sex, donor age, male patient with female donor, KPS, diagnosis, CMV serologic status, HCT-CI, HLA-match, disease risk index at transplant, and conditioning intensity were adjusted for.

Subset analysis

To identify subsets of patients who benefit most from PTCY-based prophylaxis, we performed univariable Fine and Gray regression analysis for relapse and moderate-to-severe chronic GVHD (Figure 4; supplemental Table 2). Overall, all sHRs were <1 for relapse (median sHR, 0.63; range, 0.09-0.85) indicating that PTCY was favorable across almost all subsets. Of these subsets, PTCY was particularly favorable with patient age ≥60 years (sHR, 0.61; $P = .04$), male patient sex (sHR, 0.48; $P = .018$), male patient with female donor (sHR, 0.09; $P = .016$), 8 of 8 matched PBSCT (sHR, 0.43; $P = .016$), RIC (sHR, 0.59; $P = .02$), acute myeloid leukemia (AML)/myelodysplastic syndrome (MDS) diagnosis (sHR, 0.62; $P = .05$), low/intermediate disease risk index (sHR, 0.59; $P = .036$), KPS <90 (sHR, 0.61; $P = .035$), and HCT-CI ≥ 3 (sHR, 0.53; $P = .02$). CIR curves for these subsets are presented in supplemental Figure 1. In particular, for 8-of-8 matched transplants, the CIR at 2 years was 17% in the PTCY group and 34% in the TAC group ($P = .013$); for 7-of-8 matched transplants, it was 30.8% vs 38% ($P = .34$) in the PTCY and TAC groups, respectively. (supplemental Figure 1G-H). Despite the difference in relapse, the cumulative incidence of NRM was similar for all 4 groups (2-year estimate, 11%-12%; data not shown). To further assess the effect of PTCY on conditioning regimens, we performed similar analysis for RIC and MAC regimens. In patients receiving Flu/Bu2, the 2-year CIR was 19% and 40% in the PTCY and TAC groups, respectively ($P = .01$); in patients receiving Flu/Bu4, it was 28% and 26%, respectively ($P = .97$). (supplemental Table 3; supplemental Figure 2). Of note, in patients receiving Flu/Bu2, the baseline characteristics were balanced between the TAC and PTCY groups except for a higher proportion of 7-of-8 matched transplants in the PTCY group (5.9% vs 46.2%, respectively; $P < .0001$). Furthermore, in patients with AML/MDS receiving 8-of-8 matched transplants, the 2-year CIR was 20% and 39% in the PTCY and TAC groups, respectively ($P = .027$) and 15% and 44%, respectively ($P = .015$) in patients with AML/MDS receiving 8-of-8 matched PBSCT with Flu/Bu2 regimen. (supplemental Table 3; supplemental Figure 2). However, the relapse rate was similar between the 2 groups in patients receiving reduced conditioning with Flu/Mel ($P = .77$). Relapse could not be compared in patients receiving Flu/Bu1 because no patients with Flu/Bu1 received PTCY-based prophylaxis. Because Cy is documented to have clear antineoplastic activity in lymphoma, it is noteworthy to point out that none of 7 patients with lymphoma who received PTCY relapsed (0%) whereas 20 of 48 (42%) patients with lymphoma who received TAC-based prophylaxis relapsed (2-year CIR, 0% vs 36%; $P = .03$). (supplemental Figure 3; supplemental Table 3). Because *mTOR* inhibitors could harbor antitumor activity, particularly in lymphoid neoplasms, we compared relapse rates between PTCY (n = 107) and sirolimus-containing (n = 141) GVHD prophylaxis. We found that the relapse rate was significantly lower in the PTCY

Table 1. Baseline characteristics

	TAC based (n = 463)		PTCY based (n = 107)		
	n	%	n	%	*P* value
Patient-donor characteristics					
Age, y					.11
≥60	312	67.4	63	58.9	
Median (range)	64 (21-79)		62 (21-78)		
Patient sex					.08
Female	181	39.1	52	48.6	
Male	282	60.9	55	51.4	
Donor					.051
Aged ≥40 y	42	9.1	17	15.9	
Median (range)	27 (18-54)		28 (18-64)		
Donor sex					.18
Female	161	34.8	45	42.1	
Male	302	65.2	62	57.9	
Male patient with female donor	66	14.3	17	15.9	.65
KPS					.43
100-90	162	35	33	30.8	
<90	301	65	74	69.2	
Diagnosis					.53
ALL	48	10.4	13	12.1	
AML	178	38.4	42	39.3	
MDS	128	27.6	26	24.3	
Lymphoma	48	10.4	7	6.5	
MDS/MPN	3	0.6	2	1.9	
MPN	36	7.8	8	7.5	
CML	5	1.1	3	2.8	
Other leukemia*	17	3.7	6	5.6	
Patient-donor CMV serologic status					.13
R−/D−	161	34.8	41	38.3	
R−/D+	108	23.3	29	27.1	
R+/D−	115	24.8	19	17.8	
R+/D+	79	17.1	17	15.9	
UNK			1	0.9	
HCT-CI					.28
0	16	3.5	3	2.8	
1	73	15.8	15	14	
2	61	13.2	22	20.6	
≥3	313	67.6	67	62.6	
Median (range)	4 (0-10)		3 (0-10)		
HLA type (A, B, C, or DRB1)					<.0001
8/8	436	94.2	50	46.7	
7/8	27	5.8	57	53.3	
Disease risk index					.94
Low	67	14.5	17	15.9	
Intermediate	314	67.8	70	65.4	

Matched: 8/8 matched. Mismatched: 7/8 matched.
CML, chronic myeloid leukemia; CR, complete remission; MPN, myeloproliferative neoplasm; UNK, unknown; R, recipient; D, donor.
*Other leukemia: diagnosis other than AML and MDS.
†Comparison of conditioning intensity.

Table 1 (continued)

	TAC based (n = 463)		PTCY based (n = 107)		
	n	%	n	%	*P* value
High	58	12.5	15	14	
Very high	24	5.2	5	4.7	
Transplant characteristics					
Conditioning intensity					.9†
MAC	123	26.6	29	27.1	
Flu/Bu4	100		21		
Cy/TBI	20		1		
Flu/TBI	2		7		
Flu/Bu/Mel	1				
RIC	340	73.4	78	72.9	
Flu/Bu2	270		52		
Flu/Mel	49		12		
Flu/Bu1	19				
Flu/Cy/TBI	2		14		
GVHD prophylaxis regimen					NA
PTCY/TAC/MMF			107	100	
TAC/MTX	322	69.5			
TAC/Rap	25	5.4			
TAC/Rap/MTX	116	25.1			

Matched: 8/8 matched. Mismatched: 7/8 matched.
CML, chronic myeloid leukemia; CR, complete remission; MPN, myeloproliferative neoplasm; UNK, unknown; R, recipient; D, donor.
*Other leukemia: diagnosis other than AML and MDS.
†Comparison of conditioning intensity.

group compared with sirolimus-containing GVHD prophylaxis (2-year CIR, 25% vs 41%, respectively; $P = .002$; sHR, 0.49; 95% confidence interval, 0.31-0.78) (supplemental Table 3B; supplemental Figure 3B).

For moderate-to-severe chronic GVHD, the impact of PTCY was deeper in that sHR was much lower (median sHR, 0.27; range, 0.06-0.74) and all subsets including patients who received myeloablative conditioning PBSCT (sHR, 0.08; $P = .014$) benefited substantially from PTCY-based prophylaxis with a few exceptions (Figure 4B; supplemental Table 2). The incidence rate of moderate-to-severe chronic GVHD was also significantly lower in the PTCY group (n = 107) compared with sirolimus-containing GVHD prophylaxis (n = 141) (2-year estimate, 12% vs 43%, respectively; $P < .0001$; sHR, 0.22; 95% confidence interval, 0.12-0.41).

Hematologic recovery

We assessed the impact of PTCY-based prophylaxis on neutrophil and platelet engraftment. The proportion of patients in whom neutrophils did not engraft was similar between the 2 groups (graft failure rate, 1.3% for PTCY vs 1.9% for TAC). However, the proportion of patients who never reached nadir for neutrophil count was lower in the PTCY group (0.9% vs 8%; $P = .029$; Figure 5A; supplemental Table 4). Median time to neutrophil engraftment among patients with engraftment was similar (15 and 14 days in the PTCY and TAC groups, respectively; Figure 5B; supplemental Table 4). All 8 patients who did not have engraftment died early. Platelet graft failure rate was also similar between the 2 groups (5% vs 5.6% for PTCY and TAC groups, respectively). However, the proportion of patients who never nadired for platelet count (ie, platelet count never $<20 \times 10^9$/L) was significantly lower in the PTCY group compared with that in the TAC group (4.4% vs 27.9%; $P < .0001$), despite the fact that the proportion that received RIC was well balanced between these 2 groups (73.5% vs 73.5%). In addition, median time to platelet engraftment among the patients with engraftment was delayed in the PTCY group compared with that the TAC group (23 vs 18 days; $P = .002$). (Figure 5; supplemental Table 4). Of note, HLA mismatch did not affect neutrophil or platelet engraftment.

CMV reactivation

We assessed the effect of GVHD prophylaxis regimen on the incidence of CMV reactivation requiring therapy after transplantation. For the entire cohort, the 6-month cumulative incidence of CMV reactivation requiring therapy was 9.5%. The cumulative incidence was not different between the 2 groups (9.5% vs 9.3% for PTCY and TAC, respectively; $P = .75$). The cumulative incidence rate was low when recipients were CMV seronegative (3.2% at 6 months) but high when recipients were CMV seropositive (19% at 6 months; $P < .0001$) (supplemental Table 5; supplemental Figure 4).

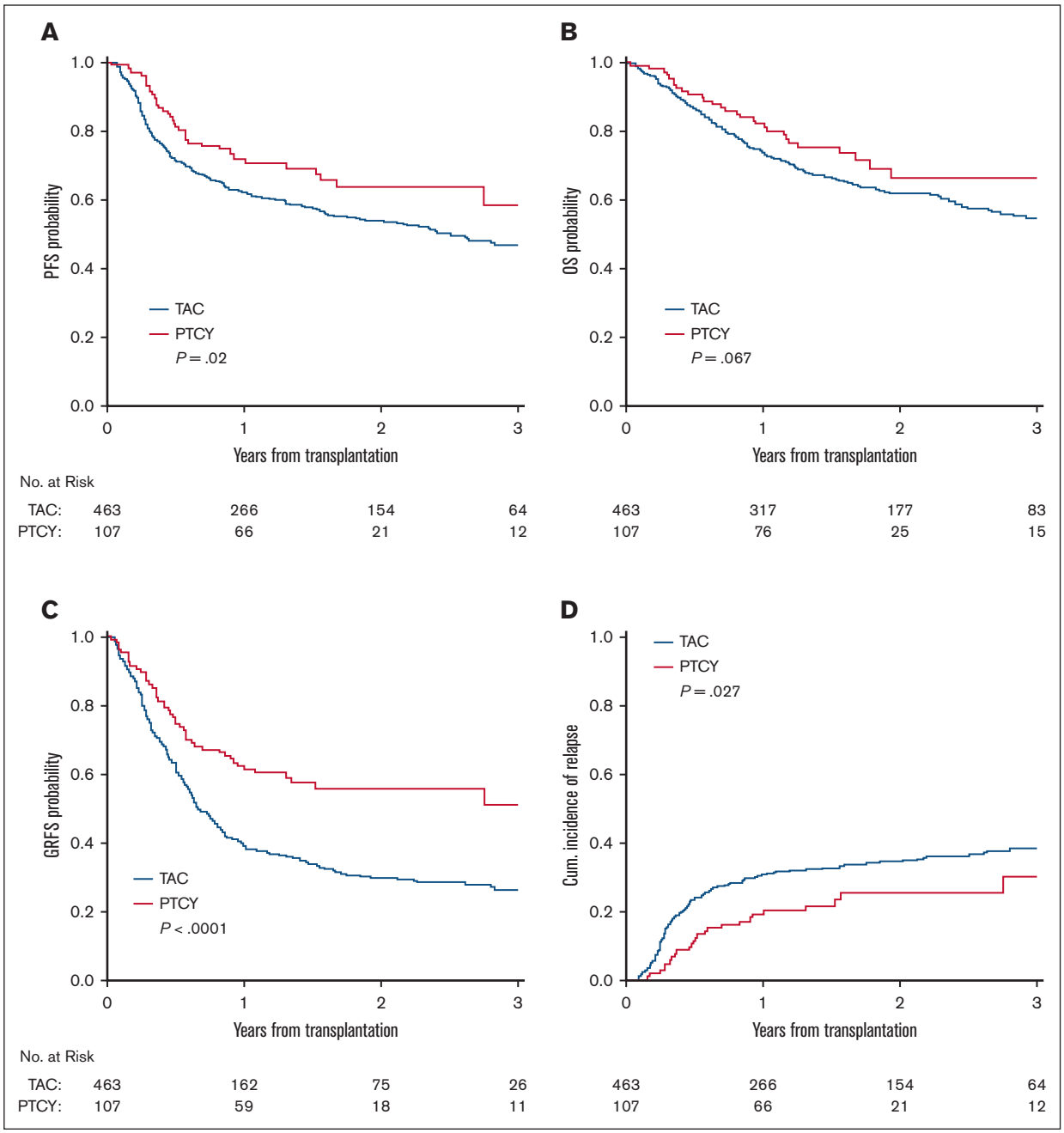

Figure 1. Kaplan-Meier curves of PFS, OS, GRFS, and CIR per prophylaxis. (A) PFS, (B) OS, (C) GRFS, and (D) CIR. Log-rank test was used for comparisons of OS, PFS, and GRFS, and Gray test was used for comparison of CIR.

Discussion

Allogeneic HCT is a well-established therapy for hematologic disorders, and its outcomes have been continuously and steadily improving over the past 40 years because of advances in donor source, conditioning regimen, high-resolution HLA typing, GVHD prophylaxis, and supportive care.[17,18] In the midst of these advances, PTCY is rapidly gaining traction for prevention of GVHD beyond the setting of haplo-HCT or mismatched URD and becoming the new standard-of-care for GVHD prophylaxis. Here, we present a real-world experience confirming favorable rates of moderate-to-severe chronic GVHD for patients receiving URD PBSCT with PTCY-based prophylaxis compared with those in patients receiving TAC-based regimens for GVHD prophylaxis (12% vs 36% at 2 years). In addition, we report a significantly lower CIR in the PTCY group compared with the TAC group (25% vs 34% at 2 years). The relapse advantage was particularly pronounced in patients who received RIC with Flu/Bu2 (19% vs 40% at 2 years; $P = .01$) and in patients with AML/MDS who received 8-of-8 matched RIC HCT (15% vs 44%; $P = .015$). This result is encouraging because disease relapse and chronic GVHD after

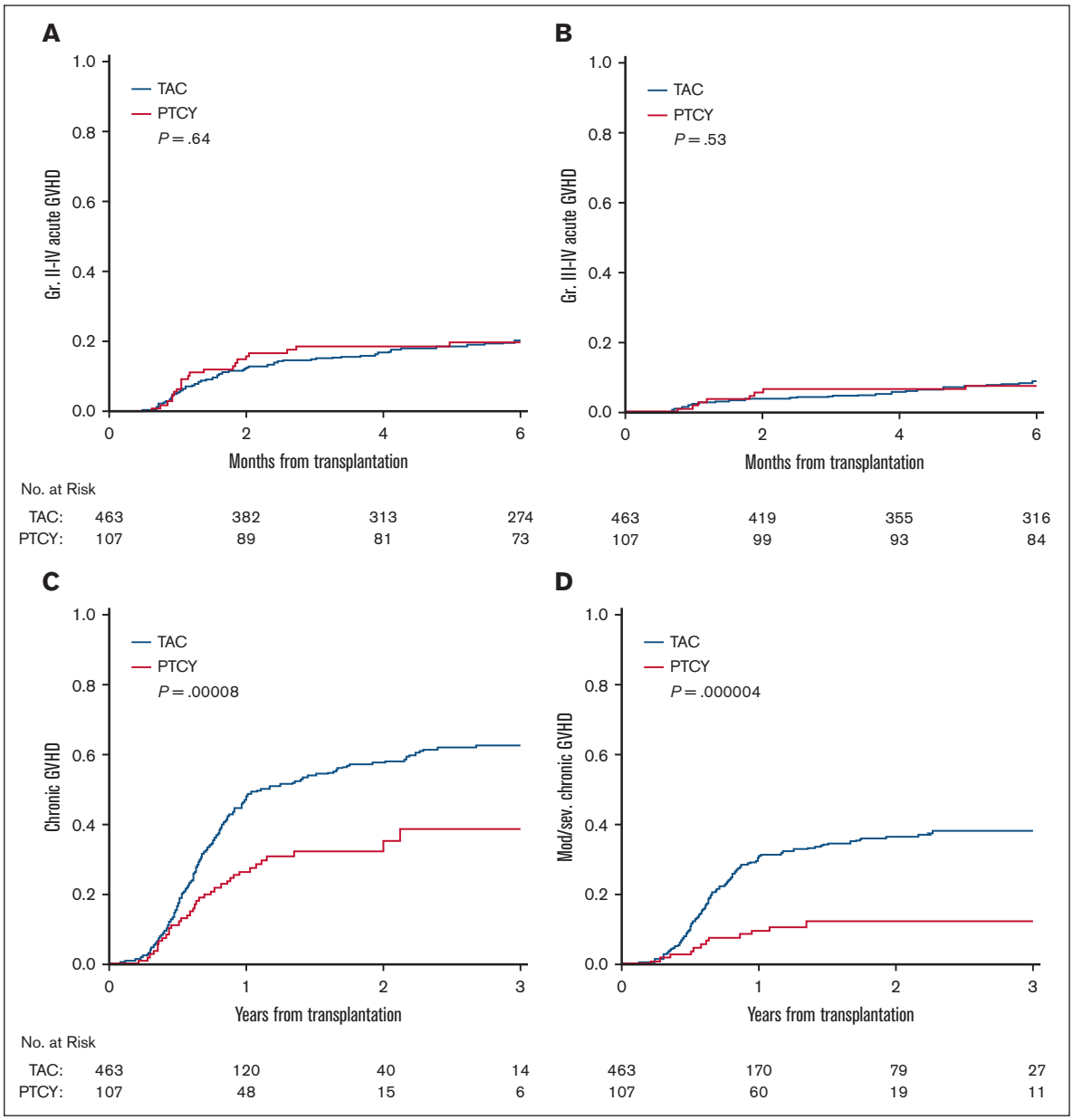

Figure 2. Cumulative incidence. (A) Grade 2-4 acute GVHD; (B) grade 3-4 acute GVHD; (C) chronic GVHD; (D) moderate-to-severe chronic GVHD.

allogeneic HCT remain major challenges, particularly in the RIC PBSCT setting. Regarding acute GVHD, the cumulative incidences of grade 2-4 (20% at 6 months in both groups) and grade 3-4 acute GVHD (4.8% vs 6.5% at day +100; 7.5% vs 8.6% at 6 months) were relatively low irrespective of the regimen and this result has been consistent at our institution over the past decade. We also assessed CMV reactivation after HCT as a surrogate measure of safety and found that the cumulative incidence rate in the PTCY group was similar compared with that in the TAC group.

A major question in transplantation is whether GVHD and GVT activity are driven by distinct immune cell subsets.[19] Mounting evidence in recent years has begun to tease apart these 2 phenomena.[20-26] Our data suggest that PTCY may play a role not only in preventing GVHD through selective depletion of alloreactive T cells but also in reducing relapse, possibly by eliminating residual tumor cells when administered with less-intensive conditioning regimens. PTCY may shift the balance of T or natural killer cell subsets in the immediate posttransplant setting away from pro-inflammatory pro-GVHD subsets and instead toward reconstitution of specific subsets that retain effective antitumor reactivity. How specific T-cell functional subsets are affected by PTCY is an area of active investigation, and future studies directed toward further dissecting these anti-GVHD and pro-GVT differences are needed.

Initially shown to suppress alloreactive T-cell subsets in murine skin allograft models,[27,28] incorporation of Cy is hypothesized to prevent chronic GVHD by eliminating activated alloreactive donor and recipient T-cell clones early after transplantation while preserving regulatory T cells.[29-31] More recent work suggests that

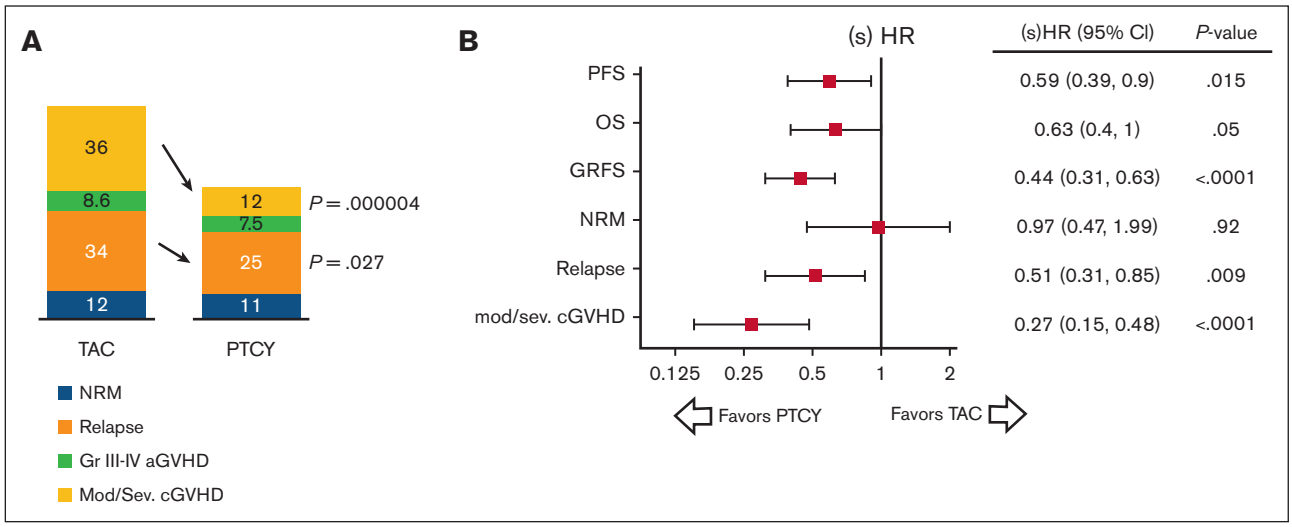

Figure 3. Summary outcome. (A) Stack plot for main outcomes. PTCY is associated with lower incidence of chronic GVHD and relapse. (B) Forest plot of (subdistribution) hazard ratios from multivariable regression analysis. Cox model was used for OS, PFS, and GRFS, and Fine and Gray model was used for NRM, relapse, and moderate-to-severe chronic GVHD.

administration of Cy before CNI abrogates the CNI effect of inhibiting T-cell exhaustion, whereas administration of CNI before transplantation fails to prevent chronic GVHD.[32] Although these mechanisms remain incompletely elucidated, use of PTCY for GVHD prevention has undergone refinements in recent years.[33] Early reports on the use of PTCY in the peritransplant period emphasized that this regimen obviated the need for T-cell depletion, preserving the GVT effect that is critical to the success of nonmyeloablative (NMA) transplantation.[34] Luznik et al later published the seminal work in haplo-HCT, demonstrating reduced chronic GVHD and acceptable rates of OS, PFS, and relapse with PTCY.[3] Based on these findings, PTCY-based regimens have been extended to the HLA-matched related or unrelated donor setting and its relative value compared with other regimens has been investigated in a series of prospective randomized trials. The results of these trials are summarized in Table 2. In randomized phase 2 (BMT CTN 1203) and 3 (BMT CTN 1703) trials comparing PTCY/TAC/MMF with TAC/MTX for patients receiving RIC PBSCT, PTCY was associated with lower incidences of severe acute and chronic GVHD but other outcomes including relapse were not different.[12,14] In these studies, various RIC regimens were allowed including Flu/Bu2, Flu/Mel, Flu/Cy, and Flu/Cy/TBI. Therefore, it was not possible to assess any association of PTCY with a particular regimen and relapse. It should be noted that in BMT CTN 1703, the majority of patients (71%) received either Flu/Mel or Flu/Cy/TBI whereas in this study, Flu/Mel comprised a minority of our study cohort and was not associated with a difference in relapse rate. In a phase 3 trial conducted by the Dutch group (HOVON-96) comparing PTCY combined with cyclosporine A to the combination of cyclosporine A and mycophenolic acid after NMA HLA-MRD or URD PBSCT, PTCY was associated with significantly lower cumulative incidences of grade 2-4 acute GVHD and extensive chronic GVHD but was not associated with other outcomes.[15] In this study, almost all patients in the PTCY arm received Flu/Cy/TBI as the conditioning regimen specified and modified from the NMA Seattle protocol.[3] In contrast, a randomized phase 3 study comparing single agent PTCY without TAC/MMF with conventional TAC/MTX GVHD prophylaxis (BMT CTN 1301) for patients receiving myeloablative conditioning and HLA-matched related or unrelated stem cells, PTCY was associated with a lower risk of relapse but other outcomes were not statistically different including acute and chronic GVHD. In this CTN study ~90% of patients in the control arm (TAC/MTX) received bone marrow stem cells rather than PBSC, perhaps underlying the observed difference. Nevertheless, PTCY was associated with less relapse compared with CNI-based prophylaxis in this randomized trial. (Table 2).

With mounting evidence of superiority of PTCY for chronic GVHD prevention in different settings, many centers, including our own, are adopting PTCY/TAC/MMF as a standard GVHD prophylaxis regimen for HLA-matched as well as HLA-mismatched donor transplants. Nevertheless, independent validation of this approach in larger multicenter studies and with analysis of real-world data is needed to clarify interactions of PTCY/TAC/MMF for GVHD prophylaxis with other critical variables such as intensity of conditioning, different conditioning regimens, and disease. This is highlighted by this study that indicates that PTCY is also associated with reduced relapse, primarily in patients who received RIC.

Our study is subject to the inherent limitations of a single-center retrospective study. Because patients were not randomly assigned to receive either PTCY vs TAC GVHD prophylaxis, it is possible that the favorable outcome in the PTCY group may be because of a selection bias, although the baseline characteristics were comparable in the 2 groups with the exception of more frequent use of PTCY in patients with HLA-mismatched donors. However, HLA disparity does not appear to account for the lower relapse rates in our study (supplemental Figure 1G-H). In the TAC group, the relapse rate was similar between 7-of-8 and 8-of-8 matched transplants (2-year rate, 38% vs 34%, respectively; $P = .51$) whereas in the PTCY group, it was actually higher for

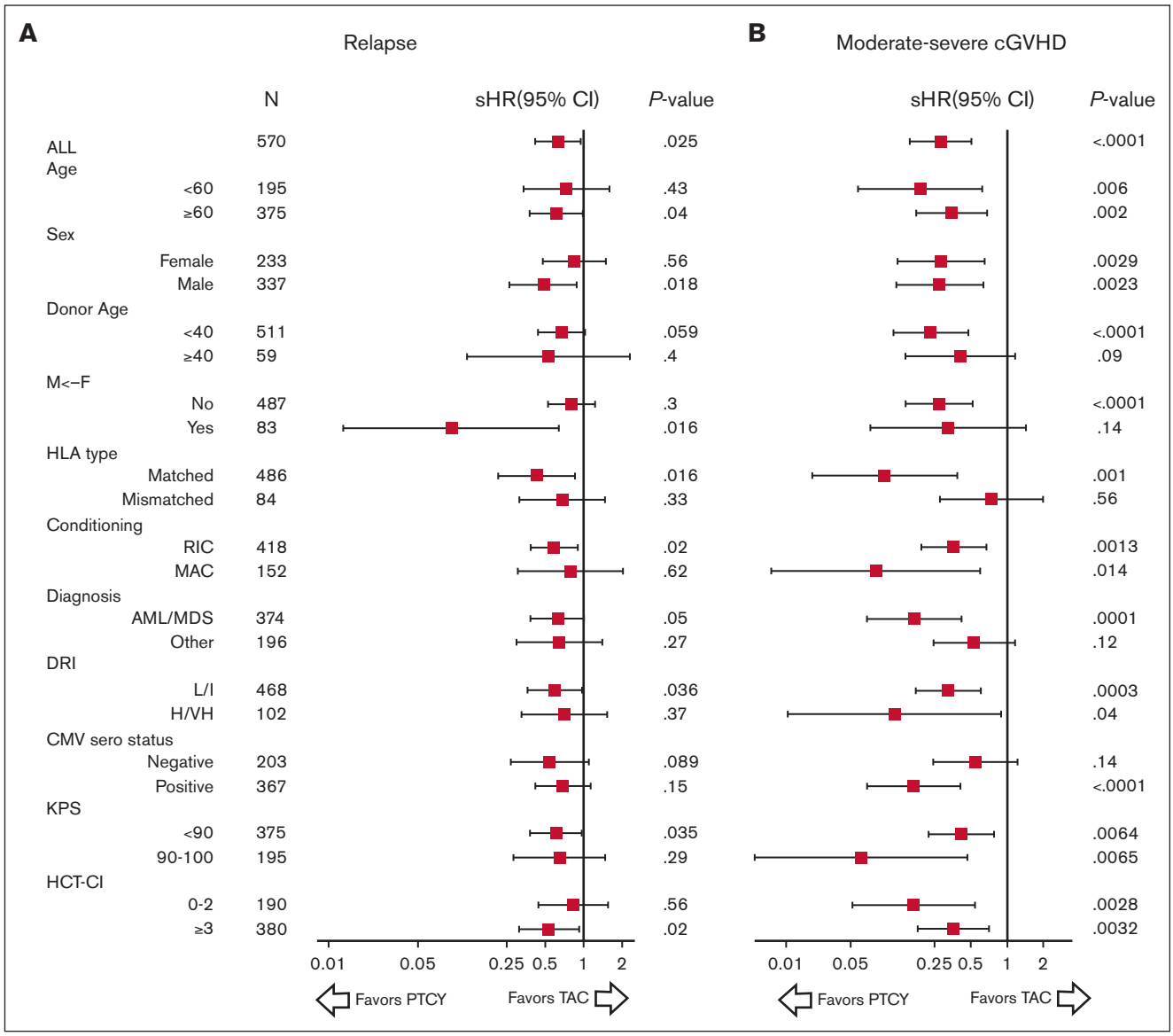

Figure 4. Forest plot of subdistribution hazard ratios from univariable Fine and Gray model for PTCY vs TAC. (A) Relapse. (B) Moderate-to-severe chronic GVHD.

7-of-8 compared with 8-of-8 matched transplants (30.8% vs 17%, respectively; $P = .12$). This finding suggests that the lower relapse seen with PCTY is unrelated to enhanced immunologic activity from HLA discrepancy. The mechanism whereby PTCY GVHD prophylaxis reduces disease relapse in our study remains to be elucidated. It is possible that, in the context of RIC, the addition of 2 doses of high-dose Cy can have a direct chemotherapeutic effect on residual tumor cells, and this translates to a relapse benefit when used in conjunction with reduced-intensity Flu/BU2 conditioning. Alternatively, it is also possible that administration of Cy in the immediate posttransplant setting may differentially deplete alloreactive T cells more than T cells capable of mediating GVT responses. Further studies are needed to understand how mechanisms of GVT effect are preserved or may even be enhanced by PTCY whereas severe GVHD is selectively suppressed. In addition, the Cy dose (50 mg/kg) used in our institution is a widely adopted dose in PTCY studies. However, whether this dose is optimal is unknown and warrants further investigation. Another limitation of our study is that measurable residual disease status at HCT was not available for the majority of patients, because high-resolution measurable residual disease flow cytometry was not consistently available at our institution in the early years of the study period. Therefore, we cannot ascertain whether the use of PTCY was able to overcome the relapse risk potentially associated with measurable residual disease positivity in these RIC transplants.

In summary, our study adds to the growing literature demonstrating that PTCY after nonhaploidentical HCT is superior to CNI/MTX in GVHD prevention without loss of GVT effect. Our study further demonstrates that relapse incidence could be lower when PTCY is used, especially in patients with AML/MDS receiving RIC with Flu/Bu2. Based on results from randomized trials, many institutions including ours are now adopting PTCY as the new standard GVHD

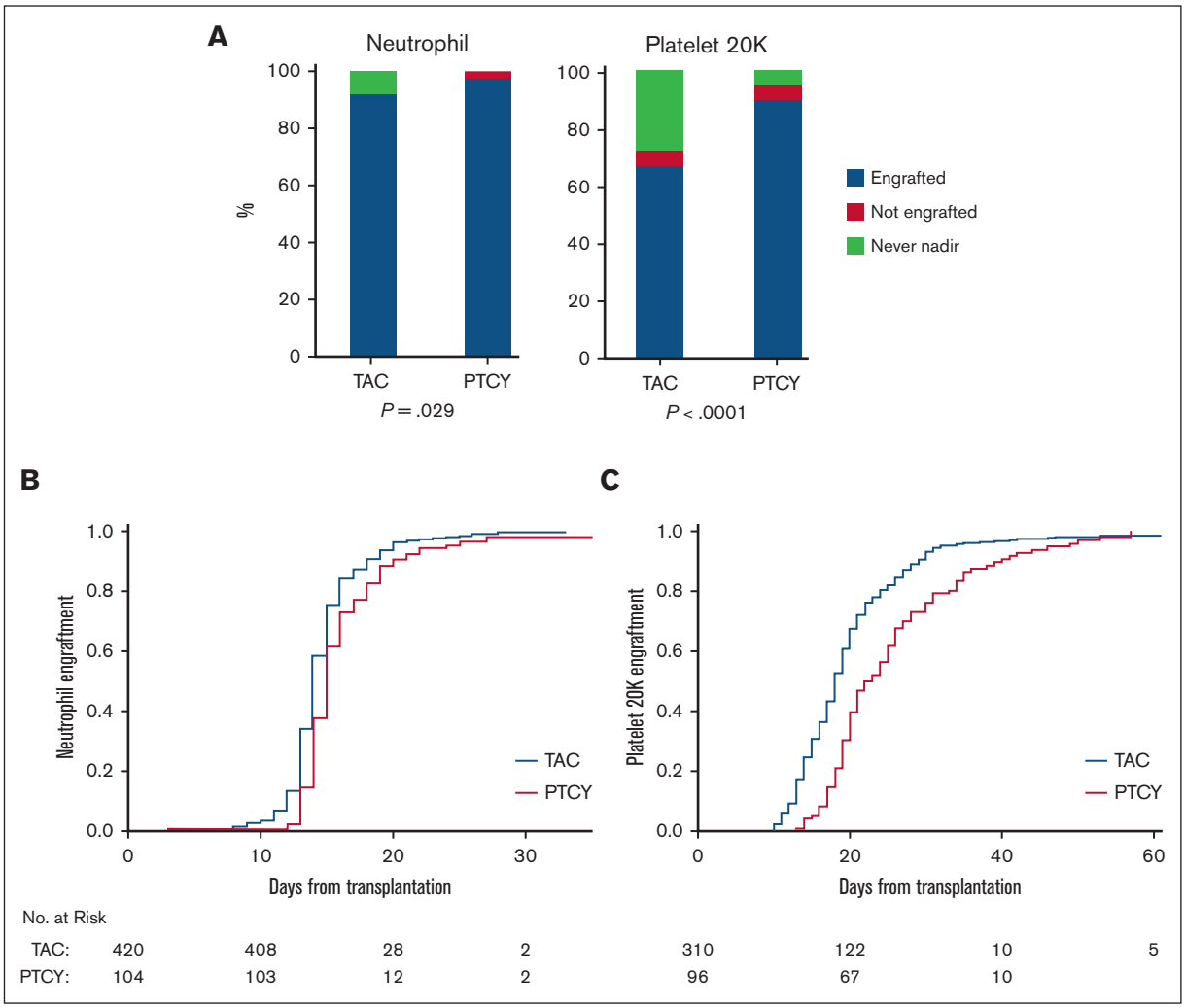

Figure 5. Neutrophil and platelet recovery. (A) The proportion of engraftment status. (B) Time to neutrophil engraftment among patients with engraftment. (C) Time to platelet engraftment among patients with engraftment.

prophylaxis regimen for matched and mismatched donor HCT. This widespread adoption should provide future opportunities to confirm and extend our observations within our, and in other, transplant centers. Future investigations are also needed to assess different preparative regimens with PTCY to identify optimal conditioning regimens for PTCY in different disease settings. Furthermore, with the recent US Food and Drug Administration approval of abatacept (ABA) as a new GVHD prophylaxis agent in mismatched donor transplantation, a prospective comparative study is needed to compare GVHD, survival, and safety between PTCY and ABA-based prophylactic regimens in various transplant strategies. Investigation on whether ABA could be incorporated with PTCY as potential future paradigms of GVHD prophylaxis should also be explored.

Acknowledgments

The authors thank the patients who participated in this trial and their families, as well as the Dana-Farber Cancer Institute/BWCC clinical oncology support staff for continued care of these patients.

This work was supported by National Institutes of Health grants P01CA229092 and P01HL158505.

Authorship

Contribution: K.M. conceived the project and wrote the manuscript; H.T.K. conceived and designed the study, performed statistical analysis, interpreted the data, and wrote the manuscript; V.T.H. edited the manuscript; E.I. collected CMV reactivation data; and all authors contributed to the manuscript review and approved the final version for submission.

Conflict-of-interest disclosure: J.R. received research funding from Equillium, Kite Pharma, Novartis, and Oncternal, and served on advisory boards for Akron, Avrobio, Clade, Erbi, Garuda, LifeVault, Novartis, Smart Immune, Talaris, and TScan. C.J.W. holds equity in BioNTech, and receives research funding from Pharmacyclics. J.K. reports research support from Amgen, Equillium, Bristol Myers Squibb, Miltenyi Biotec, Regeneron, and Clinigen; consulting income from Amgen, Equillium, and Moderna Therapeutics; and is a scientific advisory board member for Cugene and

Table 2. Summary of studies with PTCY

	Bolanos-Meads et al[12] (BMT CTN 1203)		Luznik et al[13] (BMT CTN 1301)		Holtan et al[14] (BMT CTN 1703)		Broers et al[15] (HOVON-96)		This study (Real world)	
Type of study	Randomized phase 2		Randomized phase 3		Randomized phase 3		Randomized phase 3		Retrospective	
Transplant period	2014-2016		2015-2018		2019-2021		2013-2018		2018-2021	
Graft source	PBSC		BM		PBSC		PBSC		PBSC	
Conditioning intensity	RIC		MAC		RIC		NMA		MAC, RIC	
Conditioning regimen	Flu/Bu2, Flu/Cy, Flu/Mel, Flu/Cy/TBI		Bu/Cy, Flu/Bu4, Cy/TBI, TBI/Etoposide		Flu/Mel (57%), Flu/Bu2 (27%), Flu/Cy/TBI (14%)		Flu/Cy/TBI for PTCA/CsA		Flu/Bu4 for MAC (80%) Flu/Bu2 for RIC (77%)	
Donor type	7/8-8/8 MUD, MRD		8/8 MUD, MRD		7/8-8/8 MUD, MRD		8/8 MUD, MRD		7/8-8/8 MUD	
Study arm	TAC/MTX	PTCY/TAC/MMF	TAC/MTX	PTCY	TAC/MTX	PTCY/TAC/MMF	CsA/MPA	PTCY/CsA	TAC based	PTCY/TAC/MMF
Sample size	224	92	114	109	212	208	52	99	463	107
Clinical outcome										
Grade 2-4 aGVHD	6 mo: 30%	6 mo: 27%	D100: 29.8%	D100: 37.6%	D100: 51.9%	D100: 53.8%	6 mo: 48%	6 mo: 30%*	6 mo: 20%	6 mo: 20%
Grade 3-4 aGVHD	6 mo: 13%	6 mo: 2%*	D100: 3.5%	D100: 10.1%	D100: 14.7%	D100: 6.3%*	6 mo: 12%	6 mo: 6%	6 mo: 8.6%	6 mo: 7.5%
cGVHD	1 y: 38%	1 y: 28%			1 y: 35.1%	1 y: 21.9%*	2 y: 65%	2 y: 43%*	2 y: 58%	2 y: 32%*
Mod-severe cGVHD			2 y: 33.7%	2 y: 27%	1 y: 15.6%	1 y: 6.7%*			2 y: 36%	2 y: 12%*
IS-requring cGVHD	1 y: 37%	1 y: 22%*			1 y: 25%	1 y: 12.5%*				
Extensive cGVHD							2 y: 48%	2 y: 16%*		
NRM	1 y: 16%	1 y: 11%	2 y: 7.9%	2 y: 15.7%	1 y: 17.2%	1 y: 12.3%	3 y: 14%	3 y: 10%	2 y: 12%	2 y: 11%
Relapse	1 y: 25%	1 y: 28%	2 y: 25.6%	2 y: 13.9%*	1 y: 20.2%	1 y: 20.8%	3 y: 24%	3 y: 32%	2 y: 34%	2 y: 25%*
PFS	1 y: 56%	1 y: 60%	2 y: 66.5%	2 y: 70.3%	1 y: 62.4%	1 y: 67%	3 y: 63%	3 y: 59%	2 y: 54%	2 y: 64%*
OS	1 y: 71%	1 y: 71%	2 y: 76.1%	2 y: 76.2%	1 y: 72.2%	1 y: 77%	3 y: 71%	3 y: 65%	2 y: 62%	2 y: 66%

aGVHD, acute GVHD; BM, bone marrow; cGVHD, chronic GVHD; CsA, cyclosporine A; mod-severe cGVHD, moderate-to-severe cGVHD; MPA, mycophenolic acid; IS-requiring cGVHD, chronic GVHD requiring systemic immunosuppressive treatment.

*$P < .05$, significantly different for the comparison between the PTCY arm and the control arm (TAC/MTX, CsA/MPA, or TAC based).

Therakos. S.N. reports ad hoc advisory board representation for Kite/Gilead, GlaxoSmithKline, Iovance, A2 Bio, and Sobi. R.J.S. serves on the board of directors for Be The Match/National Marrow Donor Program; provided consulting for Vor Biopharma, Neovii, CSL Behring, Bluesphere Bio, Cugene, Jasper, and Smart Immune; and is on the data safety monitoring board for Juno Therapeutics. H.T.K. provided consulting for Neovii. The remaining authors declare no competing financial interests.

ORCID profiles: K.M., 0000-0002-4160-181X; E.I., 0000-0003-0481-2404; C.J.W., 0000-0002-3348-5054; J.R., 0000-0001-5526-4669; H.T.K., 0000-0003-4889-6849.

Correspondence: Haesook T. Kim, Department of Data Science, Dana-Farber Cancer Institute, Harvard T.H. Chan School of Public Health, 450 Brookline Ave, Boston, MA 02215; email: htkimc@jimmy.harvard.edu.

References

1. Ahlmann M, Hempel G. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. *Cancer Chemother Pharmacol*. 2016;78(4):661-671.
2. Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical BMT. *Semin Oncol*. 2012;39(6):683-693.
3. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641-650.
4. Bashey A, Zhang X, Jackson K, et al. Comparison of outcomes of hematopoietic cell transplants from T-replete haploidentical donors using post-transplantation cyclophosphamide with 10 of 10 HLA-A, -B, -C, -DRB1, and -DQB1 allele-matched unrelated donors and HLA-identical sibling donors: a multivariable analysis including disease risk index. *Biol Blood Marrow Transplant*. 2016;22(1):125-133.
5. Ghosh N, Karmali R, Rocha V, et al. Reduced-intensity transplantation for lymphomas using haploidentical related donors versus HLA-matched sibling donors: a center for international blood and marrow transplant research analysis. *J Clin Oncol*. 2016;34(26):3141-3149.
6. Wachsmuth LP, Patterson MT, Eckhaus MA, Venzon DJ, Gress RE, Kanakry CG. Post-transplantation cyclophosphamide prevents graft-versus-host disease by inducing alloreactive T cell dysfunction and suppression. *J Clin Invest*. 2019;129(6):2357-2373.
7. Kanakry CG, Coffey DG, Towlerton AM, et al. Origin and evolution of the T cell repertoire after posttransplantation cyclophosphamide. *JCI Insight*. 2016;1(5):e86252.
8. Weiden PL, Flournoy N, Thomas ED, et al. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. *N Engl J Med*. 1979;300(19):1068-1073.
9. Ahmed S, Kanakry JA, Ahn KW, et al. Lower graft-versus-host disease and relapse risk in post-transplant cyclophosphamide-based haploidentical versus matched sibling donor reduced-intensity conditioning transplant for Hodgkin lymphoma. *Biol Blood Marrow Transplant*. 2019;25(9):1859-1868.
10. Sanz J, Galimard JE, Labopin M, et al. Post-transplant cyclophosphamide after matched sibling, unrelated and haploidentical donor transplants in patients with acute myeloid leukemia: a comparative study of the ALW^{P} EBMT. *J Hematol Oncol*. 2020;13(1):46.
11. McCurdy SR, Radojcic V, Tsai HL, et al. Signatures of GVHD and relapse after posttransplant cyclophosphamide revealed by immune profiling and machine learning. *Blood*. 2022;139(4):608-623.
12. Bolanos-Meade J, Reshef R, Fraser R, et al. Three prophylaxis regimens (tacrolimus, mycophenolate mofetil, and Cy; tacrolimus, methotrexate, and bortezomib; or tacrolimus, methotrexate, and maraviroc) versus tacrolimus and methotrexate for prevention of graft-versus-host disease with haemopoietic cell transplantation with reduced-intensity conditioning: a randomised phase 2 trial with a non-randomised contemporaneous control group (BMT CTN 1203). *Lancet Haematol*. 2019;6(3):e132-e143.
13. Luznik L, Pasquini MC, Logan B, et al. Randomized phase III BMT CTN trial of calcineurin inhibitor-free chronic graft-versus-host disease interventions in myeloablative hematopoietic cell transplantation for hematologic malignancies. *J Clin Oncol*. 2022;40(4):356-368.
14. Holtan SG, Hamadani M, Wu J, et al. Post-transplant cyclophosphamide, tacrolimus, mycophenolate mofetil as the new standard for graft-versus-host (GVHD) prophylaxis in reduced intensity conditioning: results from phase III BMT CTN 1703. *Blood*. 2022;140(Supplement 2):LBA-4.
15. Broers AEC, de Jong CN, Bakunina K, et al. Posttransplant cyclophosphamide for prevention of graft-versus-host disease: results of the prospective randomized HOVON-96 trial. *Blood Adv*. 2022;6(11):3378-3385.
16. Cutler C, Logan B, Nakamura R, et al. Tacrolimus/sirolimus vs tacrolimus/methotrexate as GVHD prophylaxis after matched, related donor allogeneic HCT. *Blood*. 2014;124(8):1372-1377.
17. Gooley TA, Chien JW, Pergam SA, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. *N Engl J Med*. 2010;363(22):2091-2101.
18. Canaani J, Beohou E, Labopin M, et al. Trends in patient outcome over the past two decades following allogeneic stem cell transplantation for acute myeloid leukaemia: an ALWP/EBMT analysis. *J Intern Med*. 2019;285(4):407-418.
19. Sweeney C, Vyas P. The graft-versus-leukemia effect in AML. *Front Oncol*. 2019;9:1217.
20. Slavin S, Ackerstein A, Naparstek E, Or R, Weiss L. The graft-versus-leukemia (GVL) phenomenon: is GVL separable from GVHD? *Bone Marrow Transplant*. 1990;6(3):155-161.
21. Schmid C. A step closer to GVL without GVHD. *Blood*. 2021;137(19):2565-2566.

22. Roex MCJ, Hageman L, Veld SAJ, et al. A minority of T cells recognizing tumor-associated antigens presented in self-HLA can provoke antitumor reactivity. *Blood*. 2020;136(4):455-467.

23. van Bergen CA, van Luxemburg-Heijs SA, de Wreede LC, et al. Selective graft-versus-leukemia depends on magnitude and diversity of the alloreactive T cell response. *J Clin Invest*. 2017;127(2):517-529.

24. Wang X, Schmitt A, Chen B, et al. Streptamer-based selection of WT1-specific CD8+ T cells for specific donor lymphocyte infusions. *Exp Hematol*. 2010;38(11):1066-1073.

25. Retiere C, Willem C, Guillaume T, et al. Impact on early outcomes and immune reconstitution of high-dose post-transplant cyclophosphamide vs anti-thymocyte globulin after reduced intensity conditioning peripheral blood stem cell allogeneic transplantation. *Oncotarget*. 2018;9(14):11451-11464.

26. Ikegawa S, Meguri Y, Kondo T, et al. PTCy ameliorates GVHD by restoring regulatory and effector T-cell homeostasis in recipients with PD-1 blockade. *Blood Adv*. 2019;3(23):4081-4094.

27. Eto M, Mayumi H, Tomita Y, et al. Specific destruction of host-reactive mature T cells of donor origin prevents graft-versus-host disease in cyclophosphamide-induced tolerant mice. *J Immunol*. 1991;146(5):1402-1409.

28. Huyan XH, Lin YP, Gao T, Chen RY, Fan YM. Immunosuppressive effect of cyclophosphamide on white blood cells and lymphocyte subpopulations from peripheral blood of Balb/c mice. *Int Immunopharmacol*. 2011;11(9):1293-1297.

29. Mussetti A, Greco R, Peccatori J, Corradini P. Post-transplant cyclophosphamide, a promising anti-graft versus host disease prophylaxis: where do we stand? *Expert Rev Hematol*. 2017;10(5):479-492.

30. Ganguly S, Ross DB, Panoskaltsis-Mortari A, et al. Donor CD4+ Foxp3+ regulatory T cells are necessary for posttransplantation cyclophosphamide-mediated protection against GVHD in mice. *Blood*. 2014;124(13):2131-2141.

31. Kanakry CG, Ganguly S, Zahurak M, et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. *Sci Transl Med*. 2013;5(211):211ra157.

32. Senjo H, Hashimoto D, Kubota S, et al. Calcineurin inhibitors inhibit tolerance induction by suppressing terminal differentiation of donor exhausted t cells after allogeneic SCT. *Blood*. 2022;140(Supplement 1):1159-160. Abstract 478.

33. Wachsmuth LP, Patterson MT, Eckhaus MA, Venzon DJ, Kanakry CG. Optimized timing of post-transplantation cyclophosphamide in MHC-haploidentical murine hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2020;26(2):230-241.

34. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2002;8(7):377-386.