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Post-Transplantation Cyclophosphamide and Tacrolimus for Graft-versus-Host Disease Prevention after Allogeneic Hematopoietic Cell Transplantation from HLA-Matched Donors Has More Advantages Than Limitations

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A B S T R A C T

This study compared the efficacy of graft-versus-host disease (GVHD) prophylaxis with post-transplantation cyclophosphamide (PTCy) and tacrolimus (Tac) versus other regimens in 272 adults undergoing peripheral blood (PB) allogeneic hematopoietic cell transplantation (allo-HCT) from HLA-matched donors. Of these 272 patients, 95 (34.9%) received PTCy/Tac. The times to neutrophil and platelet engraftment were longer in the PTCy/Tac group (20 days versus 16 days for neutrophils and 19 days versus 12 days for platelets). The day +30 cumulative incidence (CuI) of bacterial bloodstream infection was higher in the PTCy/Tac group (43.2% versus 13.0%; $P < .001$). The CuIs of grade II-IV and grade III-IV acute GVHD (aGVHD) at day +180 were 14.7% and 4.2%, and the CuI of moderate/severe cGVHD at 2 years was 2.4% in the PTCy/Tac group and 41.8% (hazard ratio [HR], .29; $P < .001$), 15.8%, (HR, .24; $P = .007$), and 47.0% (HR, .05; $P < .001$), respectively, in the no-PTCy group. The duration of immunosuppression was shorter in patients receiving PTCy/Tac (6.2 months versus 9.0 months; $P < .001$). PTCy/Tac patients had higher OS (2 years: 74.3% versus 60.9%; HR, .54; $P = .012$), lower NRM (2 years: 8.6% versus 15.8%; HR, .54; $P = .11$), comparable CuI of relapse (2 years: 26.0% versus 24.4%; HR, 1.03; $P = .89$),

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and higher GRFS (2 years: 59.1% versus 16.7%; HR, .32; $P < .001$). Using PTCy/Tac in HLA-matched PB allo-HCT improved transplantation outcomes at out institution compared with previous prophylactic regimens, including a higher probability of survival despite more delayed engraftment and a higher rate of bacterial infection.

INTRODUCTION

The successful use of post-transplantation cyclophosphamide (PTCy) in allogeneic hematopoietic cell transplantation (allo-HCT) performed from haploidentical (haplo-) donors [1–3] has encouraged the progressive use of PTCy for GVHD prevention with allo-HCT from HLA-matched donors, also with notable success [4–8]. However, the use of PTCy also has been associated with such undesirable outcomes as delayed engraftment, higher risk of infection, and specific organ toxicities such as cardiac complications, which raises the question of whether this prophylactic regimen should be universalized [9–11].

PTCy in combination with tacrolimus (Tac) and mycophenolate mofetil (MMF) was first implemented for GVHD prevention at our institution in patients undergoing peripheral blood (PB) haplo-HCT in 2013. Based on the positive results obtained from this prophylactic regimen, the use of PTCy was extended to allo-HCT from HLA-matched donors but with Tac as the sole complementary immunosuppressant agent (PTCy/Tac). Thus, in 2016 there was a change in practice at our institution, and PTCy/Tac became the institutional GVHD prophylaxis for allo-HCT performed from HLA-matched sibling donors (MSDs) and unrelated donors (MUDs) and using PB stem cell (PBSC) grafts [12,13].

Here we report the results of a comparative cohort study of the use of PTCy/Tac in a heterogeneous cohort of adults with different hematologic malignancies undergoing PB allo-HCT from 10/10 HLA-matched donors. The comparative cohort comprised patients who underwent transplantation at our institution prior to 2016 using non-PTCy-containing prophylactic regimens. Our findings provide additional evidence on the comparative efficacy of this alternative drug combination for GVHD prophylaxis.

METHODS

Patient Selection

This study included 272 consecutive adults with hematologic malignancies who underwent their first allo-HCT at Hospital Clínic de Barcelona between June 2011 and November 2021. All patients received PBSC grafts from an MSD or an MUD. Eligibility criteria for allo-HCT, summarized in Supplementary Data, did not differ during the study period. Retrospective data were updated in December 2022. The Hospital Clínic de Barcelona Ethics Committee approved the study, which was conducted in accordance with the principles outlines in the Declaration of Helsinki.

Conditioning Regimen, GVHD Prophylaxis, and Supportive Care

The intensity of the conditioning regimen was tailored to the patient's age and comorbidities. Myeloablative conditioning (MAC) strategies included 4 days of i.v. busulfan (Bu) 3.2 mg/kg/day in combination with 4 days of fludarabine (Flu) 40 mg/m^2/day i.v., 2 days of cyclophosphamide (Cy) 60 mg/kg/day i.v., or 12 Gy of total body irradiation (TBI). Most reduced-intensity conditioning (RIC) regimens consisted of 3 days of Bu 3.2 mg/kg/day i.v. in combination with 4 days of Flu 30 mg/m^2/day i.v. or 8 Gy of TBI. Sequential regimens were indicated in selected patients with myeloid malignancies, with combinations of Flu 150 mg/m^2, cytarabine 2 g/m^2 for 5 days, and idarubicin 12 mg/m^2 for 3 days with low-dose Bu or melphalan.

From 2011 to 2015, the institutional GVHD prophylaxis for HLA-matched allo-HCT combined a calcineurin inhibitor (Cy or Tac) with methotrexate 15 mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6, or MMF 15 mg/kg every 8 hours. No patient received antithymocyte globulin (ATG). Immunosuppressive medication was maintained at therapeutic levels until day +180 and then tapered progressively up to day +250 in the absence of GVHD.

In 2016, PTCy-Tac became our institutional GVHD prophylaxis for allo-HCT performed from 10/10 HLA MUDs, and in 2019, its use was extended to allo-HCT performed from MSDs [14,15]. All consecutive patients who underwent transplantation beyond these dates received PTCy-Tac for GVHD prevention. PTCy was administered at a dose of 50 mg/kg/day IV and on days

+3, and +4 in all cases. Tac was initiated at a dose of .04/kg/24 hours i.v. on day +5, maintained at therapeutic levels until day +90, and tapered progressively up to day +180 in the absence of GVHD. The calcineurin inhibitor administration route was switched from i.v. to oral between day +12 and +20 after stem cell engraftment.

All patients received unmanipulated T cell-replete PBSC grafts with a maximum CD34 cell dose of 8×10^6. G-CSF was not used during the study period in accordance with protocol. No patient received letermovir for cytomegalovirus (CMV) prevention, as its use had not yet been approved in Spain. CMV reactivation necessitating preemptive intervention was established at a CMV viremia of >1000 copies/mL of CMV-DNA in plasma. CMV disease was defined as PCR-confirmed CMV replication from tissue biopsy in patients with clinical suspicion. Acute GVHD (aGVHD) was graded according to the MAGIC (Mount Sinai Acute GVHD International Consortium) criteria [16], and chronic GVHD (cGVHD) was graded based on the 2014 National Institutes of Health Consensus Criteria [17]. Patients with GVHD were managed homogeneously during the study period. Additional definitions are summarized in the Supplementary Data.

Statistical Analysis

The main explanatory variable of interest was the type of GVHD prophylaxis (PTCy/Tac versus other). Primary outcome variables were the cumulative incidence (CuI) of GVHD and the post-transplantation variables overall survival (OS), nonrelapse mortality (NRM), and GVHD-free/relapse-free survival (GRFS).

The study cohort was divided into 2 groups according to the GVHD prophylaxis regimen. Categorical variables were presented as count and percentage; continuous variables, as median and range. Post-transplantation follow-up was censored at 2 years to homogenize patient follow-up, permitting a more precise analysis of the impact of implementing PTCy/Tac on OS and GRFS. The CuI of GVHD was estimated considering death and relapse as competing events. OS, RFS, and GRFS were calculated using the Kaplan-Meier product-limit method [18]. GRFS was calculated accounting for death, relapse, grade III-IV aGVHD, and moderate/severe cGVHD as events [19]. The NRM and CuI of relapse (CIR) were estimated using the CuI method. Relapse was considered a competing event in the analysis of NRM, and death without relapse was considered a competing event in the study of CIR [14].

The impacts of the main explanatory variable (ie, type of GVHD prophylaxis) on the probabilities of GVHD, OS, and GRFS were explored using multivariable Cox regression analyses [15] to control for other risk factors. The baseline risk factors included in each multivariable model were selected based on clinical judgment before the analysis. The primary study variable GVHD prophylaxis (ie, PTCy/Tac versus other) was included as an explanatory variable in all estimations. The models of GVHD prediction included the covariate constellation of age at allo-HCT (continuous), infusion of grafts from female donors to male recipients (yes versus no); time period (2017-2020 versus 2011-2016), and the presence of grade III-IV aGVHD (time-dependent variable included only in the model with the dependent variable cGVHD). The models for prediction of OS included the covariates age (continuous), Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI; >3 versus 0-3), Karnofsky Performance Status (<90 versus 90-100), Disease Risk Index (DRI; high/very high versus low/intermediate), donor type (MUD versus MSD), conditioning intensity (RIC versus MAC), and the presence of grade III-IV aGVHD (time-dependent variable) and any cGVHD (time-dependent variable). The model for GRFS included the covariates age (continuous), HCT-CI (>3 versus 0-3), Karnofsky Performance Status (<90 versus 90-100), DRI (high/very high versus low/intermediate), donor type (MUD versus MSD), and conditioning intensity (RIC versus MAC). All P values were 2-sided, and the level of statistical significance for the estimated values of the coefficients of the explanatory variables was set at $P < .05$. The statistical analysis and estimations were performed using EZR [20].

RESULTS

Baseline Information

The median age of the 272 patients included in the study was 53 years (range, 18 to 70 years). The cohort had a male preponderance (55.9%; n = 152), and the most prevalent baseline diagnoses were acute myeloid leukemia (38.2%) and myelodysplastic syndrome (18.0%). One hundred twenty-three patients (45.2%) received an MSD graft, and 149 (54.8%) received an MUD graft. Ninety-five patients (34.9%) received PTCy/Tac for GVHD prophylaxis.

As reported in Table 1, the baseline characteristics were balanced between the 2 study groups, except for a higher proportion of patients age >60 years (18.9% versus 6.8%; P = .002) and a higher proportion of MUD allo-HCT (82.1% versus

Table 1
Patient and Transplantation Characteristics

Characteristic	PTCy/Tac Group (N = 95)	No-PTCy Group (N = 177)	*P*
Age, yr, median (range)	54 (22-70)	53 (18-69)	.986
Age ≥60 yr, n (%)	18 (18.9)	12 (6.8)	.002
Female sex, n (%)	39 (41.1)	81 (45.8)	.456
Baseline diagnosis, n (%)			-
Acute myeloid leukemia	35 (36.8)	69 (39.0)	
Myelodysplastic syndrome	18 (18.9)	31 (17.5)	
Myeloproliferative Disorders	9 (9.5)	10 (5.6)	
Acute lymphocytic leukemia	19 (20.0)	25 (14.1)	
Lymphoproliferative disorder	10 (10.6)	29 (15.4)	
Chronic myeloid leukemia	1 (1.1)	9 (5.1)	
Prolymphocytic leukemia	3 (3.2)	4 (2.3)	
HCT-CI >3	29 (30.5)	33 (22.9)	.189
KPS 70-80, n (%)	22 (23.2)	37 (27.6)	.448
DRI, n (%)			
Low-intermediate	66 (71.8)	115 (66.9)	.416
High-very high	26 (28.3)	57 (33.1)	
Not applicable	3	5	
CMV risk status, n (%)			
Low	14 (14.7)	18 (10.2)	.546
Intermediate			
High	27 (28.4)	50 (28.2)	
Donor type, n (%)			
10/10 MSD	17 (17.9)	106 (59.9)	<.001
10/10 MUD	78 (82.1)	71 (40.1)	
Female donor to male recipient, n (%)	11 (11.6)	28 (15.8)	.341
Conditioning regimen intensity, n (%)			
Myeloablative	44 (46.3)	81 (45.8)	.930
Reduced intensity	51 (53.7)	96 (54.2)	
Conditioning regimen (extended), n (%)			
Bu/Cy	0	28 (15.8)	
Cy/TBI (12 Gy)	0	24 (13.6)	
Flu/TBI (12 Gy)	19 (6.9)	5 (2.8)	
Flu/TBI (8 Gy)	6 (2.2)	0	
Flu/Bu (4)	22 (8.0)	20 (11.3)	
Flu/Bu (3)	38 (13.4)	56 (31.6)	
Flu/melphalan	0	25 (14.1)	
Thiotepa/Bu/Flu	4 (1.4)	3 (1.7)	
Sequential RIC allo-HCT	5 (1.8)	13 (7.3)	
Other	1 (.4)	3 (1.7)	
Follow-up, mo, median (IQR)	23.5 (15.6-24)	24 (7.5-24)	.78

KPS indicates Karnofsky Performance Status.
*Post-transplantation follow-up was censored at 2 years.

40.1%) in the PTCy/Tac group. Considering that this study included consecutive adult allo-HCT recipients over a period of 10 years, the post-transplantation follow-up was homogeneously censored at 2 years.

Post-Transplantation Information and Infectious Complications

As described in Table 2, 270 patients (99.3%) achieved engraftment. The median time to neutrophil engraftment (20 days versus 16 days;

Table 2
Post-Transplantation Data and Outcomes

Parameter	PTCy/Tac Group (N= 95)	No-PTCy Group (N = 177)	*P* Value
Transplantation hospitalization, d, median (IQR)	37 (32-43)	30 (28-36)	<.001
Post-transplantation complications, n (%)			
Sinosoidal obstruction syndrome	1 (1.1)	8 (4.5)	.168
Thrombotic microangiopathy	1 (1.1)	12 (6.8)	.038
Engraftment			
Time to neutrophil engraftment, d, median (IQR)	20 (18-24)	16 (14-18)	.001
Time to platelet engraftment, b, median (IQR)	19 (13-27)	12 (10-15)	.001
Primary graft failure, n (%)	1 (1.1)	1 (.5)	
CuI of infectious complications, % (95% CI)			
Day +30 bacterial BSI	43.2 (33.0-52.9)	13.6 (8.5-18.4)	<.001
Day +180 CMV reactivation	45.0 (33.8-55.6)	59.1 (51.0-66.3)	.144
Day +180 CMV disease	5.3 (1.9-11.1)	7.9 (4.5-12.5)	.233
Day +180 Epstein-Barr virus reactivation	1.1 (.1-5.2)	0	.958
Day +180 grade 2-4 BK virus HC	12.6 (6.9-20.2)	6.8 (3.7-11.1)	.134
CuI of GVHD, % (95% CI)			
Grade II-IV aGVHD at day +180	14.7 (8.5-22.6)	41.8 (8.5-22.6)	<.001
Grade III-IV aGVHD at day +180	4.2 (1.4-9.7)	15.8 (10.9-21.6)	.003
cGHVD at 2 yr	17.1 (9.8-26.1)	54.2 (45.7-61.9)	<.001
Moderate/severe cGVHD at 2 yr	4.4 (1.4-8.5)	45.9 (37.7-53.8)	
Additional information, % (95% CI)			
Day +180 CuI of cardiac toxicity	9.5 (4.6-16.4)	4.0 (1.8-7.6)	.068
Day +180 CuI of ICU admission	12.6 (6.9-20.2)	7.9 (4.5-12.5)	.198
Time to IS discontinuation, mo, median (IQR)	6.3 (5.8-7.9)	9.0 (5.8-19.6)	<.001
Main outcome information, n (%)*			
Relapse (at 2 yr)	27 (28.4)	58 (32.7)	.461
Mortality (at 2 yr)	22 (23.1)	69 (38.9)	.008
Main post-transplantation outcomes, % (95% CI)*			
OS			.011
1 yr	83.8 (74.6-89.9)	66.1 (58.6-72.6)	
2 yr	74.3 (63.4-82.3)	60.9 (53.3-67.7)	
RFS			.107
1 yr	69.3 (58.9-77.5)	55.9 (48.3-62.9)	
2 yr	62.2 (51.4-71.2)	53.1 (45.4-60.1)	
NRM			.078
1 yr	8.6 (4.0-15.4)	14.5 (9.7-20.3)	
2 yr	8.6 (.4-15.4)	15.8 (10.8-21.8)	
CuI of relapse			.893
1 yr	21.3 (13.6-30.2)	23.1 (17.1-29.7)	
2 yr	26.0 (17.5-35.4)	24.4 (18.2-31.1)	
GRFS			<.001
1 yr	66.2 (55.6-74.7)	26.6 (20.3-33.2)	
2 yr	59.1 (48.3-68.3)	16.7 (11.6-22.6)	

CI indicates confidence interval; IS, immunosuppression.
* Post-transplantation follow-up was censored at 2 years.

$P < .001$) and platelet engraftment (19 days versus 12 days; $P < .001$) were longer in the PTCy/Tac group. Two patients (.7%) experienced primary graft failure (GF), 1 of whom had received PTCy/Tac. The 2 patients with primary GF were previously diagnosed with myelodysplastic syndrome and primary myelofibrosis, underwent a second allograft, and engrafted. However, both patients died secondary to complications derived from the second allo-HCT.

The transplantation-related hospital stay was longer in patients receiving PTCy/Tac (37 days versus 30 days; $P < .001$). The percentage of patients with veno-occlusive disease was similar in the 2 groups (1.1% versus 4.5%; $P = .168$). The incidence of post-transplantation thrombotic microangiopathy was lower in the PTCy/Tac group (1.1% versus 6.8%; $P = .038$). A non-statistically significant trend toward a higher incidence of cardiac toxicity was observed in the PTCy/Tac group (day +180: 9.5% versus 4.0%; $P = .068$). Furthermore, the difference in ICU admission rate between the 2 study groups was not statistically significant (day +180 CuI of ICU admission, 12.6% versus 7.9%; $P = .198$).

Sixty-three patients (23.1%) had a first bacterial bloodstream infection (BSI) episode during the first 30 days after allo-HCT. The day +30 CuI of BSI was 43.2% for patients receiving PTCy/Tac and 13.0% for those who did not ($P < .001$). The day +30 CuIs of gram-positive and gram-negative BSI were 14.7% and 24.2%, respectively, for the PTCy/Tac group and 10.2% ($P = .323$) and 2.8% ($P < .001$) for the no-PTCy group. The day +30 mortality rate attributed to the first episode of BSI was 1.6%, with no differences between the 2 study groups. One hundred thirty-two patients (48.5%) experienced CMV reactivation, and 5 (1.8%) had CMV disease. No patient received letermovir. The day +180 CuI of CMV reactivation was 45.0% for the PTCy/Tac group and 59.1% for the no-PTCy group ($P = .144$). No patient received letermovir. Twenty-two patients (8.1%) had CMV disease, with a day +180 CuI of 5.3% for the PTCy/Tac group and 7.9% for the no-PTCy group receiving other prophylaxis ($P = .233$). Patients were started on either ganciclovir/valganciclovir or foscarnet. Although all patients had an initial clinical response, 8 patients (36.6%) died during the first 100 days after the diagnosis of CMV disease, 6 patients secondary to transplantation-related toxicity, including a concomitant documentation of aGVHD or a bacterial BSI, and 2 patents with previous disease relapse. Grade 2-4 BK-positive hemorrhagic cystitis (HC) was diagnosed in 22 patients (8.1%). The day +180 CuI for HC was 12.6% for the PTCy/Tac group and 6.8% for the non-PTCy/Tac group ($P = .134$). The day +100 mortality rate among patients with HC was 18.1% (n = 4 patients).

GVHD

aGVHD was diagnosed in 130 patients (47.7%). The maximum grade achieved was I in 40 patients (14.7%), II in 57 patients (20.9%), and III-IV in 33 patients (12.1%). As described in Table 2 and Figure 1, the day +180 CuI of grades II-IV and III-IV aGVHD were 14.7% and 4.2%, respectively, in patients receiving PTCy/Tac, and 41.8% ($P < .001$) and 15.8% ($P = .003$) in the no-PTCy/Tac group. The median time to the diagnosis of aGVHD in the 2 groups was 39 days (IQR, 27 to 46 days) and 34 days (IQR, 22 to 57 days), respectively ($P = .819$). Clinical manifestations did not differ between the 2 study groups. Twelve patients (4.4%) died secondary to steroid-refractory aGVHD, most of them with a concomitant infection; only 1 of these patients received PTCy/Tac. cGVHD occurred in 94 patients (34.5%). the maximum grade of cGVHD was mild In 23 of these patients (8.4%), moderate in 46 (16.9%), and severe in 25 (9.1%). The 2-year CuI of cGVHD was 17.1% for the PTCy/Tac group and 54.2% for the no-PTCy/Tac group. Similarly, the 2-year CuI of moderate/severe cGVHD was lower in the PTCy/Tac group (2.4% versus 47.0%; $P < .001$) (Figure 1). The median time to cGVHD diagnosis was 7.5 months (IQR, 6.3 to 8.7 months) in the PTCy/Tac group and 8.5 months (IQR, 5.6 to 8.7 months) in the no-PTCy/Tac group ($P = .318$), and the median time to moderate/severe cGVHD diagnosis in the 2 groups was 8.8 months (IQR, 6.3 to 11.8 months) and 5.7 months (IQR, 5.3 to 6.2 months), respectively ($P = .218$). All patients were started on active treatment, and only 1 patient (.4%), from the no-PTCy/Tac group, died secondary to severe and refractory multiorgan cGVHD during the first 2 years after allo-HCT.

The results of multivariable analysis reported in Table 3 confirms that when controlling for variables to be relevant determinants of GVHD, the average incidence of grade II-IV aGVHD (hazard ratio [HR], .43; $P = .017$), grade III-IV aGVHD (HR, .33; $P = .08$), and moderate/severe cGHVD (HR, .05; $P < .001$) were lower in the PTCy/Tac group. The results from Table 3 also show that infusing grafts from female donors to male patients tends to increase the risk for grade III-IV aGVHD (HR, 2.07; $P = .052$).

Duration of Immunosuppression and Immune Reconstitution

Following predefined institutional clinical practice, the tapering of immunosuppression was started earlier in the patients who received PTCy/Tac. Thus, the duration of the immunosuppression was shorter in these patients (median, 6.2 months versus 9.0 months; $P < .001$) (Table 2). T cell immune reconstitution data were evaluated only in alive patients with at least 1 year of post-

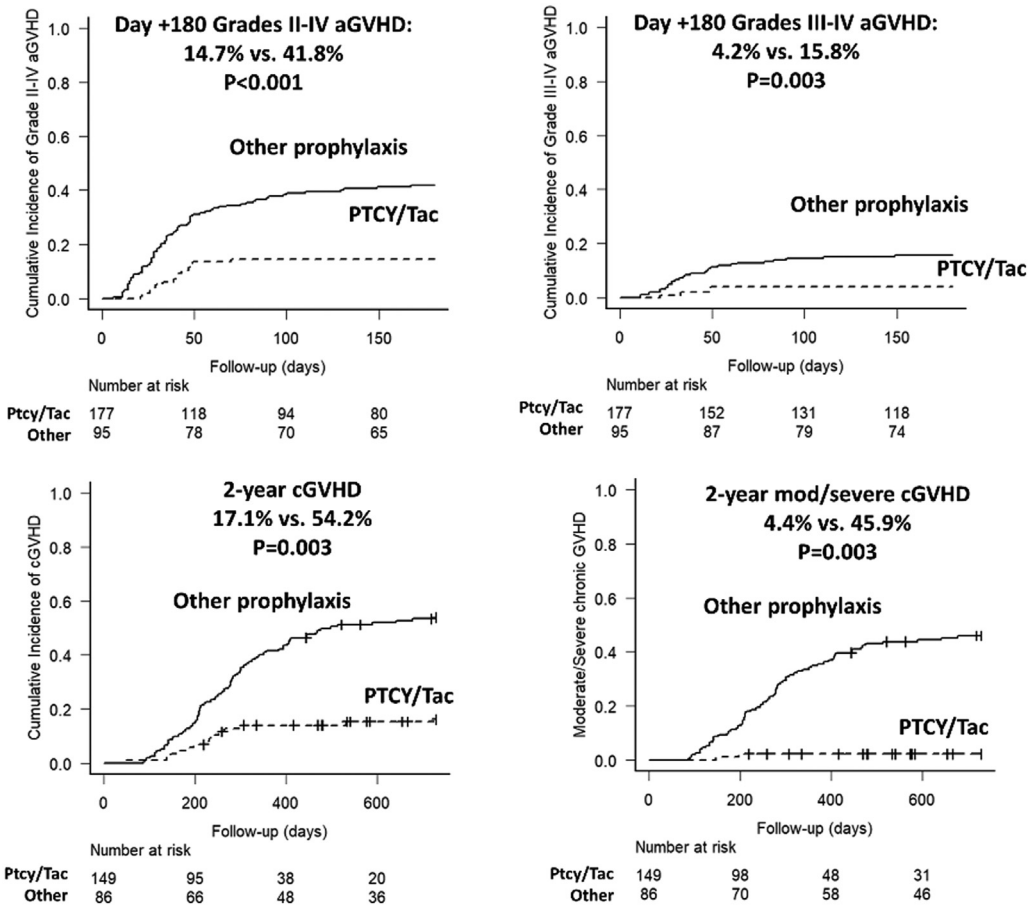

Figure 1. Incidences of aGVHD and cGVHD according to prophylaxis regimen.

transplantation follow-up and without disease relapse; among them, data were available for 100 patients from the PTCy/Tac group and 97 patients in the no-PTCy/Tac group. The median times to CD4 and CD8 cell dose recovery (>200 cells/mm^2) were 7.6 and 5.6 months, respectively, in the PTCy/Tac group and 8.6 (P = .789) and 7.8 (P = .135) months in the no-PTCy/Tac group.

Disease Relapse and Post-Transplantation Outcomes

Data on the primary outcome are reported in Table 2 and Figure 2. Eighty-seven patients (32.0%) relapsed during the first 2 years after allo-HCT. The 2-year CIR was 26.0% in the PTCy/Tac group and 24.4% in the no-PTCy/Tac group (HR, 1.03; P = .89). The median time to disease

Table 3
Predictors of aGVHD and cGVHD

Multivariate Model	Grade II-IV aGVHD, HR (95% CI)	*P*	Grade III-IV aGVHD, HR (95% CI)	*P*	Moderate/ Severe cGVHD, HR (95% CI)	*P*
GVHD prophylaxis, PTCy/Tac vs other regimens	.43 (.21-.87)	.017	.33 (.09-1.14)	.08	.05 (.01-.22)	<.001
Age, continuous	.98 (.96-1.01)	.060	1.01 (.97-1.02)	.900	1.01 (.98-1.02)	.837
Donor/recipient sex, female/male recipient vs other	1.21 (.71-2.07)	.470	2.07 (.99-4.33)	.052	.91 (.47-1.77	.792
Time period, 2018-2020 vs 2014-2017	.57 (.33-.98)	.043	.69 (.28-1.69)	.420	.70 (.39-1.25)	.239
Grade II-IV aGVHD, time-dependent variable	N/A		N/A		1.01 (.63-1.60)	.980

N/A, not applicable.

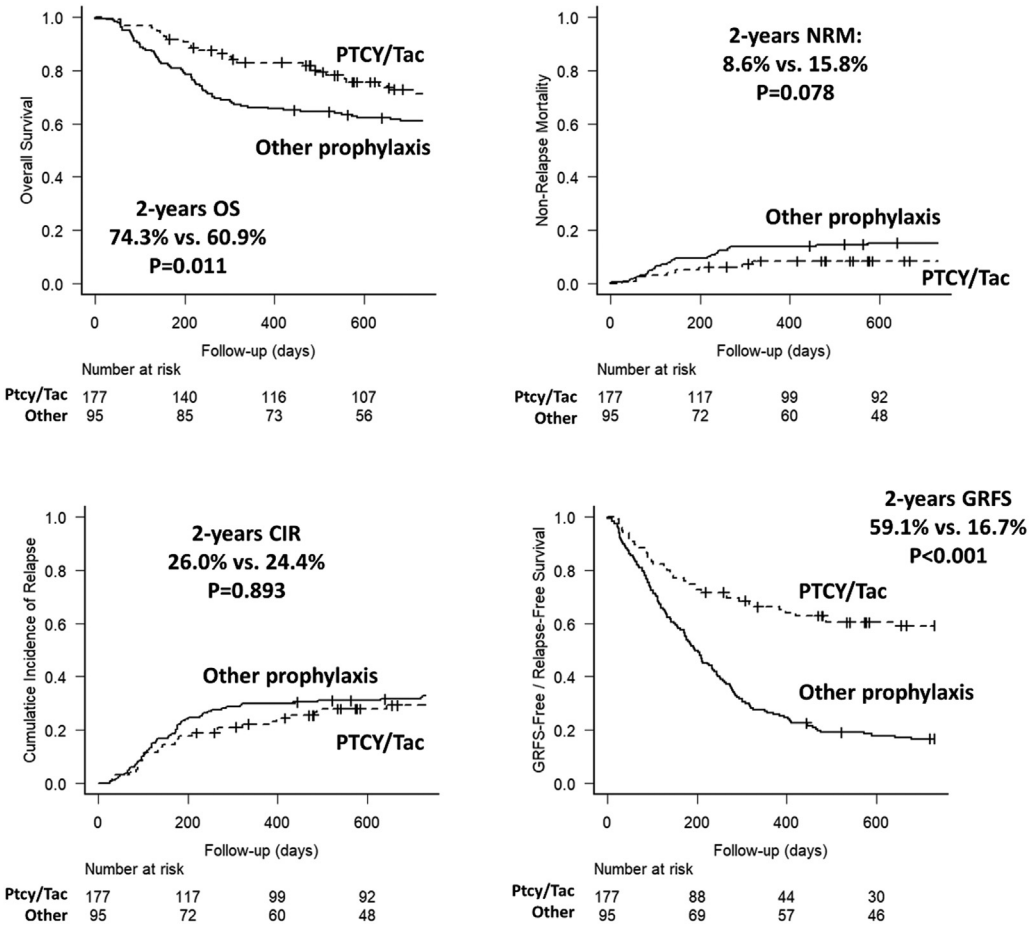

Figure 2. Main post-transplantation outcomes according to GVHD prophylaxis regimen.

relapse in the 2 groups was 4.8 months (IQR, 3 to 9.7 months) and 5.3 months (IQR, 3.2 to 8.2 months) (P = .948).

Ninety-four patients (34.5%) died during the first 2 years after allo-HCT, with relapse and infection the main causes. As shown in Figure 2, patients in the PTCy/Tac group had a higher 2-year OS (74.3% versus 60.9%; HR, .54; P = .011), a higher but not statistically significant 2-year RFS (62.2% versus 53.3%; HR, .72; P = .108), a nonsignificant trend toward lower 2-year NRM (8.6% versus 15.8%; HR, .52; P = .11), and a higher 2-year GRFS (59.1% versus 16.7%; HR, .32; $P < .001$) compared with patients receiving other prophylaxis.

The estimated probabilities of OS and of GRFS are shown in Table 4. On multivariable analysis, the probability of OS was higher in the PTCy/Tac group (HR, .54; P = .031), as was the probability of GRFS (HR, .40; $P < .001$) (Figure 2). Furthermore, OS was lower in patients with an HCT-CI >3 (HR, 1.80; P = .022) and a high or very high DRI (HR, 1.79; P = .015). Patients with grade III-IV aGVHD also had a lower probability of OS (HR, 5.17; $P <$ .001), but the probability of OS was higher in patients with cGVHD than in those without cGVHD (HR .16; $P < .001$).

DISCUSSION

The main result of this study is that PTCy, administered at a dose of 50 mg/kg on days +3 and +4 and followed by Tac, induces effective prevention of aGVHD and cGVHD when PBSC grafts from HLA-matched donors are used for allo-HCT. The use of this prophylaxis permits rapid discontinuation of immunosuppression during the post-transplantation period. These findings are notable because clinically relevant aGVHD was associated with a higher probability of mortality in our study, and the diagnosis of GVHD has a negative impact on patients' quality of life [21,22].

In 2016, PTCy/Tac was implemented in our institution for PBSC allo-HCT from 10/10 HLA-matched and single-loci HLA-mismatched unrelated donors. Since then, 3 single-arm retrospective analyses have evaluated the efficacy of PTCy/Tac prophylaxis. Two of these studies demonstrated that PTCy/Tac induced sufficient immunosuppression to allow engraftment and effectively

Table 4
Risk Factors for OS and GRFS

Variable	OS, HR (95% CI)	*P*	GRFS, HR (95% CI)	*P*
Univariate model				
GVHD prophylaxis, PTCy-Tac vs other regimens	.54 (.33-.87)	.013	.32 (.22-.46)	<.001
Age, continuous	1.01 (.98-1.02)	.781	1.01 (.99-1.01)	.680
Age >60 yr	.96 (.61-1.52)	.877	.96 (.70-1.32)	.811
HCT-CI >3 vs 0-3	1.55 (.96-2.49)	.067	1.07 (.75-1.53)	.702
KPS <90 vs 90-100	1.55 (.94-2.55)	.082	1.16 (.80-1.67)	.422
DRI high/very high vs low/intermediate	1.76 (1.15-1.69)	.008	1.68 (1.24-2.28)	<.001
Donor type, MUD vs MSD	.85 (.57-1.29)	.461	.60 (.45-.81)	<.001
Conditioning regimen intensity, RIC vs MAC	.98 (.65-1.48)	.928	1.09 (.82-1.46)	.531
Grade II-IV aGVHD, time-dependent variable	2.02 (1.33-3.06)	.009	N/A	
Grade III-IV aGVHD, time-dependent variable	4.70 (2.92-7.56)	<.001	N/A	
Any cGVHD, time-dependent variable	.27 (.12-.62)	.001	N/A	
Multivariate model				
GVHD prophylaxis, PTCy-Tac vs other regimens	.54 (.30-.94)	.031	.40 (.27-.60)	<.001
Age, continuous	1.02 (.99-1.05)	.082	1.01 (.99-1.02)	.528
KPS <90 vs 90-100	1.67 (1.01-2.81)	.049	1.08 (.74-1.57)	.676
HCT-CI >3 vs 0-3	1.80 (1.07-3.02)	.022	1.11 (.76-1.63)	.580
DRI high/very high vs low/intermediate	1.79 (1.08-2.95)	.015	1.55 (1.09-2.21)	.014
Donor type, MUD vs MSD	1.07 (.64-1.88)	.791	.81 (.57-1.15)	.251
Conditioning regimen intensity, RIC vs MAC	.60 (.33-1.26)	.103	1.06 (.67-1.66)	.794
Grade III-IV aGVHD, time-dependent variable	5.17 (2.75-9.71)	<.001	N/A	
Any cGVHD, time-dependent variable	.16 (.05-.47)	<.001	N/A	

prevent GVHD [12,23]. Consecutively, a third retrospective and comparative analysis demonstrated that PTCy/Tac was safe and provided superior GVHD prevention than other prophylaxis without ATG in adults age >50 years who underwent transplantation from an HLA-matched or 9/10 HLA-mismatched donor [13]. That study compared the efficacy of PTCy/Tac in adults undergoing HLA-matched PBSC HCT and reported positive results.

Few studies to date have investigated the efficacy of double-PTCy-based prophylaxis in HLA-matched PBSC allo-HCT [24–27]. One of the first such publications reported prospective data from PTCy and cyclosporine (CsA) use in 43 patients undergoing MAC PBSC allo-HCT from 8/8 HLA-matched donors [26]. The authors observed a high incidence of grade II aGVHD (77%), although with no cases of severe aGVHD and a low incidence of cGVHD (15%) [26]. Another prospective study conducted in Japan concluded that PTCy/Tac in 10/10 HLA-matched PBSC allo-HCT provided effective GVHD prevention, with reported incidences of grade II-IV and III-IV aGVHD and cGVHD of 18%, 5.9%, and 12%, respectively, in the MSD group and 18%, 9.1%, and 9.1% in the MUD group [25]. Although these findings are encouraging, the replication of these results in other cohorts of patients potentially could be limited because the study sample was composed mainly of Asian patients. More recently, a retrospective analysis including a large, heterogeneous cohort of 242 adults found incidence rates of 38% for grade II-IV aGVHD and 8% for grade III-IV aGVHD at day +180 and 8% for 2-year moderate/severe cGVHD [27], in line with the incidence rates found in our present study. Interestingly, that study also compared the effectiveness of PTCy/Tac with PTCy/Tac/MMF and concluded that the addition of MMF did not provide any advantage for GVHD prevention in this setting [27]. Finally, a prospective study published in 2022 (HOVON trial) found that in recipients of allo-HCT performed from 8/8 HLA-matched donors, GVHD prevention was better in patients receiving PTCy/CsA than in patients receiving conventional prophylaxis [6]. The 6-month incidences of grade II-IV and III-IV aGVHD were 30% and 6% in the PTCy/CsA group and 48% and 12% in the CsA/MMF group. Two-year rates of extensive cGVHD in the 2 groups were 16% versus 48%, and rates of GRFS at 1 year were 45% and 21%.

In our cohort, the likelihood of OS was higher in the PTCy/Tac group compared with the no-PTCy group when controlling for other factors affecting

the risk of mortality. The results are in line with those reported by the few studies that have evaluated the efficacy of double PTCy-based prophylaxis on HLA-matched allo-HCT [24–27]. Also remarkable is that by reducing clinically relevant GVHD and improving OS, PTCy/Tac was associated with significantly increased GRFS at 2 years (59.1% versus 16.7%). In other words, at 2 years after the procedure, 59% of the patients with hematologic malignancies who underwent allo-HCT from an HLA-matched donor and received GVHD prophylaxis with PTCy/Tac at our institution were alive, in remission, and without clinically relevant GVHD at the time of this report [28].

A positive association between cGVHD and OS was observed in our analysis. A protective effect of cGVHD on the probability of OS has been observed in previous studies, in which cGVHD has been associated with the graft-versus-leukemia effect and lower relapse risk [29–33]. However, our results must be considered with caution, given that the post-transplantation follow-up of our patients was censored at 2 years. Given that cGVHD-associated NRM is a late post-transplantation event, the limited post-transplantation follow-up of our analysis might have resulted in underestimation of the impact of clinically relevant cGVHD in long-term NRM.

Remarkably, despite the fact that PTCy/Tac decreased the incidence of cGVHD, and that cGVHD was associated with lower OS, the observed survival rate was higher in patients who received PTCy/Tac. Furthermore, similar to other reports [6,34-36], the lower cGVHD incidence observed in the PTCy-based group did not result in an increased risk of relapse. Our explanation for this is that the extra cytotoxic effect induced by PTCy and more rapid discontinuation of the immunosuppression observed in patients receiving PTCy/Tac may enhance the graft-versus-leukemia effect and effectively prevent disease relapse.

PTCy/Tac was associated with delayed neutrophil and platelet recovery, a higher incidence of day +30 BSI, and prolonged transplantation-related hospitalization. In addition, high incidences of CMV reactivation and disease and grade-2-4 BK-positive HC were documented, although without statistically significant differences from patients who received traditional GVHD prophylaxis. For these reasons, the introduction of PTCy/Tac prophylaxis demands more human and nonhuman resources and likely increases the cost of treatment. At the same time, the reduction in cGVHD has reduced the number of medical appointments needed during long-term follow-up, which has saved human and nonhuman resources. An evaluation of the net economic impact of implementing PTCy/Tac is underway at out institution. Furthermore, there have been recent changes to our allo-HCT platform, including the use of letermovir prophylaxis for CMV-seropositive recipients and the administration of G-CSF from day +7 to the time of neutrophil recovery, aimed at minimizing transplantation-related toxicity.

We also examined the T cell immune reconstitution time and found similar estimated median times to CD4 and to CD8 recovery in the 2 study groups. This result contrasts with the positive association between the use of PTCy and delays in immune reconstitution found in previous studies [9,10,37-39]. We hypothesize that the association of PTCy with only 1 immunosuppressant agent, together with the rapid discontinuation of immunosuppression, accelerated T cell immune reconstitution to the point where the median recovery time became similar in the 2 groups.

The retrospective study design and differences in post-transplantation follow-up time between the 2 study groups were considered the main limitations of the present analysis. To overcome the second limitation, the post-transplantation follow-up period was censored at 2 years for all patients. Furthermore, the fact that the control arm did not include patients receiving ATG precluded a comparison of the effectiveness of PTCy/Tac and ATG-based prophylaxis. Nevertheless, considering the limited data published supporting the feasibility of using double PTCy-based prophylaxis in PBSC HLA-matched allo-HCT [24–27], the results of this study should be of interest for the allo-HCT community.

CONCLUSION

The present study shows that the double-based prophylaxis with PTCy/Tac established at our institution in 2016 has proven safer and more effective than other GVHD prophylaxis regimens without ATG used in PBSC allo-HCT from HLA-matched donors. Furthermore, post-transplantation OS and GRFS rates have improved at our institution since the advent of combination PTCy/Tac GVHD prophylaxis.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2023.11.020.

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