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PTCY and Tacrolimus for GVHD Prevention for Older Adults Undergoing HLA-Matched Sibling and Unrelated Donor AlloHCT

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A B S T R A C T

The use of post-transplantation cyclophosphamide (PTCY) for graft-versus-host disease (GHVD) prevention is becoming prevalent in the transplantation community when HLA-identical sibling and 10/10 HLA-matched (MUD) and 9/10 mismatched unrelated donors are selected for alloHSCT. However, reported evidence on outcomes from elderly patients receiving PTCY-containing GVHD prophylaxis remains limited. This study aims to compare the outcomes of PTCY- tacrolimus (TK) prophylaxis and conventional GVHD prophylaxis in patients aged >50 years undergoing peripheral blood alloHSCT from a single institution. A total of 161 consecutive patients aged >50 years undergoing alloHSCT between January 2014 and February 2021 were included. Data were collected retrospectively and updated in December 2021. Patients received grafts from HLA-identical sibling, and from 10/10 and 9/10 HLA matched and mismatched unrelated donors. Overall, median age was 60 years, and 91 (54.8%) received PTCY-TK for GVHD prevention. Time to neutrophil and platelet engraftment was longer in the PTCY-TK group (20 versus 16 days and 19 versus 11 days, $P < .001$). The cumulative incidences of grade II-IV and III-IV acute GVHD (aGVHD) at day 100 and moderate/severe chronic GVHD (cGVHD) at 2 years were 18.2%, 5.7%, and 9.5% for patients receiving PTCY-TK, and 26.0%, 9.6% and 39.5% for those who did not. The multivariate analysis showed that PTCY-TK reduced the probability of grade II-IV aGVHD (hazard ratio [HR] 0.41, $P = .035$), of cGVHD (any grade: HR 0.43 [$P = .014$], and of moderate/severe cGVHD [HR 0.15 {$P < .001$}]). At 2 years, the overall survival (65.4% versus 65.6%, $P = .472$), non-relapse mortality (17.4% versus 13.7%, $P = .967$), and cumulative incidence of relapse rates (24.2% versus 27.5%, $P = .712$) were comparable between both cohorts; GVHD-free/relapse-free survival (GRFS) was higher in the PTCY-TK group (2 years: 50.2% versus 21.8%; HR 0.42, $P = .001$). In patients aged ≥50 years. PTCY-TK was safe and a more effective drug combination than non-PTCY containing GVHD prophylaxis, even with the use of matched and mismatched unrelated donors, and resulted in comparable relapse rates and better GRFS.

The last decade has witnessed a remarkable reduction in graft-versus-host disease (GVHD) rates, secondary to introducing new methods for GVHD prevention [1]. The use of post-transplantation cyclophosphamide (PTCY) to mitigate bidirectional alloreactivity has been a milestone in haploidentical hematopoietic stem cell transplantation (haploHSCT) [2–4]. Consequently with its efficacy, the use of PTCY is being extended to HLA-identical sibling (MSD) and 10/10 HLA-matched (MUD) and 9/10 mismatched (MMUD) unrelated donors allogeneic HSCT (alloHSCT) [5–8].

Improvements in induction therapies and in supportive care have resulted in an increasing number of older patients with high-risk hematological disorders achieving disease remission and being considered for alloHSCT. But, despite the

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advances in curative potential, historically the transplant-related mortality rate after alloHSCT has been higher in the older than in the younger population [9,10]. The use of PTCY is becoming prevalent in the transplant community; however, reported evidence on outcomes from elderly receiving PTCY-containing GVHD prophylaxis is still limited.

Between 2013 and 2015, the use of PTCY-based immunoprophylaxis was progressively implemented at our institution independently of the selected donor source. Based on the experience gained from the use of PTCY in this setting, and under the hypothesis that less immunosuppression would be sufficient to permit engraftment and prevent GVHD, since 2016 PTCY combined with tacrolimus (PTCY-TK) alone has been the regular GVHD prophylaxis for MSD and UD alloHSCT at our institution, with notable success [11,12]. The present study investigates whether PTCY-TK prophylaxis provides better outcomes in terms of safety and effectiveness than conventional GVHD prophylaxis in patients of age 50 and older who underwent alloHSCT from MSD and from unrelated donors (UD).

METHODS

Patient Selection

A total of 161 patients with hematological malignancies aged 50 and older who consecutively underwent their first alloHSCT between January 2014 and February 2021 at the Hospital Clinic de Barcelona, Spain, were included in the study. All patients received peripheral blood stem cell (PBSC) grafts from MSD and UD (10/10 HLA MUD and 9/10 MMUD). Clinical information was collected retrospectively and updated in December 2021. According to the GVHD prophylaxis, the study cohort was stratified into 2 groups: PTCY-TK and others. The study was approved by the Ethics Committee of the Hospital Clinic de Barcelona and conducted following standards set forth by the Declaration of Helsinki.

Conditioning Regimens and Graft-versus-Host Disease Prophylaxis

The intensity of the conditioning regimen (myeloablative [MAC] versus reduced-intensity [RIC]) was tailored according to the patient's age, performance status, and comorbidities. The most frequent MAC strategies were fludarabine 40 mg/m^2/d intravenously (IV) x 4 days combined with busulfan 3.2 mg/Kg/day IV $\times$ 4 days, or 8 and 12 Gy of total body irradiation. In the case of RIC transplants, the most prevalent conditioning regimen consisted of fludarabine 30 mg/m^2/day IV $\times$ 4 days combined with busulfan 3.2 mg/Kg/d IV $\times$ 3 days or melphalan 70 mg/m^2 IV $\times$ 2 days.

PTCY-based GVHD prophylaxis consisted of the administration of 50 mg/kg/d of PTCY IV on days 3 and 4, followed by TK initiated at a dose of 0.03/kg/24 h IV on day 5. TK was titrated to achieve a 5 to 15 mg/mL therapeutic level. Mycophenolate mofetil (MMF) was not administered to any of the patients included in the PTCY-TK arm. Other GVHD prophylaxis combined calcineurin inhibitors (cyclosporine [CsA] or TK) with standard doses of methotrexate, MMF, or sirolimus (SIR). Anti-thymocyte globulin (ATG) or alemtuzumab was not administered. Immunosuppression was generally maintained in therapeutic ranges until day 90 and then tapered if GVHD grade II-IV was absent.

Graft Characteristics and Supportive Care

T-cell replete PBSC grafts were infused in all cases; the maximum CD34+ cell dose infused on day 0 was capped at 8 $\times$ 10^6/kg. Granulocyte colony-stimulating factor was not administered per protocol. Standard infectious prophylaxis consisted of levofloxacin 500 mg daily until neutrophil engraftment; fluconazole 400 mg daily from the start of conditioning and continued for 2 months; acyclovir 800 mg twice daily for 12 months; and either trimethoprim-sulfamethoxazole 160/800 mg 3 times per week or inhaled pentamidine 300 mg monthly until the achievement of peripheral blood CD4+ cell counts $>$ 200 cells/μL. None of the patients received letermovir as cytomegalovirus (CMV) prophylaxis.

Statistical Method

GVHD prophylaxis was considered the study's primary explanatory variable of interest (PTCY-TK versus others). Primary outcome measures were the cumulative incidences of acute GVHD (aGVHD) and chronic GVHD (cGVHD), overall survival (OS), and GVHD-free/relapse-free survival (GRFS). Other outcomes of interest were non-relapse mortality (NRM), relapse-free survival (RFS), and cumulative incidence of relapse (CIR).

Descriptive variables were calculated using counts and percentages, and continuous variables were reported with median and ranges. Time to event was calculated from the date of the transplantation to the date of the event or last follow-up. OS, RFS, and GRFS were calculated using the Kaplan-Meier method. GRFS was calculated accounting for death, relapse, grade II-IV acute GVHD, and moderate/severe cGVHD events. NRM and CIR were estimated using the competing risk analysis and considering relapse as a competing event for NRM and death without relapse as a competing event for CIR. The cumulative incidence of GVHD was calculated accounting for death and relapse as competing events, and the calculation of cumulative incidence of infectious complications accounted for death as a competing event. Univariate and multivariate regression tests explored the impact of the primary variable of interest (GVHD prophylaxis) on post-transplantation outcomes. Those variables found to be significant in the univariate analysis and considered clinically relevant were all included in the multivariate regression models. All P values were 2-sided, and a P value $<$ .05 was considered to indicate statistical significance. Statistical analysis was performed using EZR software [13].

RESULTS

Patient Information

Characteristics of patients in each group of GVHD prophylaxis are summarized in Table 1. Overall, the median age was 60 years, 114 (70.8%) patients underwent RIC alloHSCT, and 88 (54.7%) received PTCY-TK for GVHD prevention. Baseline characteristics of study participants were balanced between the 2 cohorts with different prophylaxis, except for the proportions of males (63.6% versus 45.2%; P = .026) and of patients receiving UD grafts (93.3% versus 42.4%; P $<$ .001) that were higher in the PTCY-TK arm. Additionally, the donor's median age was lower in the PTCY-TK arm (33 versus 48 years, P $<$ .001). The proportion of alloHSCT performed using PTCY-TK was higher after 2017 (63.6% versus 24.7%, P $<$ .001), and consequently, the median follow-up of this group was shorter (18.8 versus 37.1 months; P = .027).

Engraftment Information

Of the 161 patients included, 159 (98.7%) primary engrafted. The median of days for neutrophil and platelet engraftments was 20 (18-25) and 19 (12-30) days in the PTCy arm compared with 16 (15-18) and 11 (9-13) days in the non-PTCY group (differences statistically different). Donor chimerism measured in either CD3+ or unsorted cells was found to be $>$95% in 145 of the 151 patients (96.0%) and in 112 of the 118 (94.9%) measured patients, respectively, by day 30 and 60 after the stem cell infusion.

Two (1.3%) patients had primary graft failure. These patients received grafts from 9/10 MMUD (HLA disparity in locus A and B, respectively) and PTCY-TK. Donor chimerism at day 30 was, respectively, 0% and 4%. No patient developed HLA donor-specific antibodies. The 2 patients underwent second alloHSCT using alternative donor sources and successfully engrafted. However, both patients died complications derived from the second transplant.

Main Post-Transplantation Complications and Infectious Rates

No patient had cytokine release syndrome. Noninfectious-related fever after PBSC infusion was documented in 5 (3.1%) patients. Two patients received grafts from MMUD. Post-infusion fevers resolved in 24 to 48 hours and had no impact on post-transplantation morbidity and mortality. Rates of grade 3-4 mucositis were higher in patients receiving PTCY-TK (35.2% versus 19.1%, P = .024). Seventeen (10.2%) patients required therapeutic parenteral nutrition, and 12 (13.1%) of them received PTCY-TK (13.6% versus 6.8%, P = .163). Four (2.4%) recipients developed moderate-severe hepatic sinusoidal obstruction syndrome, and one of them received PTCY-TK (1.1% versus 4.1%, P = .228). All patients recovered after fluid restriction, diuretics, and defibrotide. Transplant admission was longer for patients receiving PTCY-PK than for patients who did not (median days: 37 versus 30, P $<$ .001).

Table 1
Baseline Characteristics

	All Patients N = 161 (100%)	PTCY-TK N = 88 (54.7%)	Other GVHD Prophylaxis N=73 (45.3%)	*P* Value
Age at alloHSCT median, years (range)	60 (50-70)	60 (50-70)	59 (50-69)	.879
Older than ≥ 65 years	39 (24.2)	26 (29.5)	13 (17.8)	.080
Male sex	89 (55.3)	56 (63.6)	33 (45.2)	.026
Baseline Diagnosis				–
AML	58 (36.0)	31 (35.2)	27 (37.0)	
MDS/CMML	51 (31.8)	28 (31.8)	23 (31.5)	
NPM	15 (9.3)	6 (6.8)	7 (9.6)	
ALL	9 (5.6)	11 (12.5)	3 (4.1)	
NHL/CLL	21 (13.0)	12 (13.6)	9 (12.3)	
Others	7 (4.3)	3 (3.4)	4 (5.5)	
Disease Risk Index				.585
Low-Moderate	101 (65.2)	57 (64.8)	44 (60.3)	
High-Very High	54 (34.8)	28 (31.8)	26 (35.6)	
Non-Classifiable/Missing	6	3	3	
Karnofsky Performance Status 60-80%	36 (22.4)	21 (23.9)	15 (20.5)	.615
HCT-CI score >3	41 (25.5)	21 (23.9)	20 (27.4)	.608
Donor selection				<.001
HLA MSD	48 (28.9)	6 (6.6)	42 (56.0)	
10/10 HLA MUD	75 (46.5)	45 (51.1)	39 (40.0)	
9/10 HLA MMUD	43 (26.7)	40 (46.5)	3 (4.0)	
HLA mismatch				
A	21 (13.0)	21 (23.9)	0	
B	9 (5.6)	9 (10.2)	0	
C	4 (2.5)	4 (4.5)	0	
DR or DQ	9 (5.6)	6 (6.8)	3 (4.0)	
Donor age				<.001
Median years (IQR)	38 (27-53)	33 (25-41)	48 (34-59)	
Older 50 years	44 (27.3)	9 (10.2)	35 (47.9)	
Intensity of the conditioning regimen				.914
Myeloablative	47 (29.2)	26 (29.5)	21 (28.8)	
Reduced Intensity	114 (70.8)	62 (70.5)	52 (71.2)	
Conditioning regimen				–
Myeloablative				
Cy-Bu (4)	5 (3.1)	0	5 (6.8)	
Flu-Bu (4)	30 (18.6)	18 (20.4)	13 (17.8)	
Flu-TBI (12Gy)	4 (2.5)	3 (3.4)	1 (1.4)	
Flu-TBI(8Gy)	4 (2.5)	4 (4.5)	0	
TBF	3 (1.9)	1 (1.1)	2 (2.7)	
Reduced Intensity				
Flu-Bu3	105 (65.2)	59 (67.0)	45 (61.7)	
Flu/Mel	8 (5.0)	1 (1.1)	7 (9.6)	
Cy/Flu/TBI (2Gy)	2 (1.2)	2 (2.3)	0	
CD34 cell dose: median $\times 10^6$ (range)	5.59 (2.1-19.8)	5.70 (2.1-9.5)	5.54 (2.3-19.8)	.284
Range of Time				<.001
2014-2017	87 (54.0)	32 (36.4)	55 (75.3)	
2018 - 2021	74 (46.0)	56 (63.6)	18 (24.7)	
Median follow-up: median months (range)	25.4 (0.9-89.8)	18.8 (0.9-68.4)	37.1 (2.6-89.8)	.027

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; CLL, chronic lymphocytic leukemia; Bu, busulfan; Flu, fludarabine; TBI, total body irradiation; TBF, thiotepa-busulfan-fludarabine; Mel, melphalan.

As shown in Table 2, bacterial bloodstream infections (BSI) were more prevalent in patients receiving PTCY-TK (Day 180 cumulative incidences of 16.5% and 4.3% (P = .015), respectively). The cumulative incidences of CMV reactivation (Day 180: 47.4% versus 56.2%, P = .614) and of CMV disease (Day 180: 5.7% versus 6.8%, P = .791) were comparable between the 2 cohorts. CMV disease was diagnosed in 13 (8.0%) patients, with the gastrointestinal tract the most prevalent organ affected. Eight (61.5%) of 13 patients received PTCY-PK. Three patients died with active CMV infection, 2 of them from steroid-refractory aGVHD and one secondary to a septic shock. The cumulative incidence of Epstein-Barr virus reactivation at day 180 was 1.1% for patients receiving PTCY-TK and 0 for those receiving other GVHD prophylaxis (P = .312). No patient had post-transplantation lymphoproliferative disorders.

Table 2
Main Post-Transplant Information According to GVHD Prophylaxis

	All Patients N = 161 (100%)	PTCY-TK N = 88 (54.7%)	Other GVHD Prophylaxis N = 73 (45.3%)	*P* Value
Post-transplantation Information				
Engraftment information				
Median days neutrophil engraftment (IQR)	18 (16-22)	20 (17-25)	16 (15-18)	<.001
Median days platelet engraftment (IQR)	13 (11-21)	19 (12-30)	11 (9-13)	<.001
Graft failure	4 (2.4)	4 (4.5)	0	.127
Primary	2 (1.2)	2 (2.3)	0	—
Cumulative incidence infectious complications, % (95% CI)				
Day 180 bacterial bloodstream infection	10.5 (6.1-16.2)	16.5 (8.9-26.0)	4.3 (1.1-11.1)	.015
Day 180 CMV reactivation	51.6 (43.5-59.0)	47.7 (36.9-57.7)	56.2 (43.9-66.7)	.614
Day 180 CMV disease	6.2 (3.2-10.7)	5.7 (2.1-7.9)	6.8 (2.5-14.2)	.791
Day 180 EBV reactivation	0.6 (0.1-3.2)	1.1 (0.1-5.6)	0	.312
Day 180 Grade 2-4 BK virus hemorrhagic cystitis	7.5 (4.1-12.2)	11.4 (5.8-19.0)	2.7 (0.5-8.6)	.038
Day 180 other viral infection	21.1 (15.2-27.7)	22.7 (14.6-32.0)	19.2 (11.1-29.0)	.853
Day 180 pulmonary fungal infection (probable and proven)	4.3 (1.9-8.3)	4.5 (1.5-10.4)	4.1 (1.1-10.5)	.927
Cumulative incidence of GVHD, % (95% CI)				
Day 100 Grade II-IV aGVHD	21.7 (15.7-28.4)	18.2 (10.9-26.9)	26.0 (16.6-36.5)	.103
Day 100 Grade III-IV aGVHD	7.5 (4.1-12.2)	5.7 (2.1-11.9)	9.6 (4.2-17.7)	.240
2-year any grade cGVHD	40.8 (32.6-48.9)	29.2 (19.0-40.1)	52.1 (39.7-63.1)	.006
2-year moderate/severe cGVHD	24.1 (17.4-31.3)	9.4 (4.0-17.6)	39.5 (28.0-50.8)	<.001
Median months to immunosuppression discontinuation (IQR)*	9.1 (6.4-13.9)	7.8 (6.2-10.5)	13.2 (9.1-26.0)	<.001
Main post-transplantation outcomes				
Relapse	44 (27.3)	22 (25.0)	22 (30.1)	.467
Death	58 (36.0)	28 (31.8)	30 (41.1)	.222
Causes of death†				
Relapse	30 (18.6)	13 (14.8)	17 (23.3)	—
Infection	10 (6.2)	9 (10.2)	1 (1.4)	
GVHD	11 (6.8)	1 (1.1)	10 (13.7)	
Graft failure	3 (1.9)	3 (3.4)	0	
Secondary malignancy	2 (1.2)	2 (2.3)	0	
Hemorrhage	2 (1.2)	0	2 (2.7)	
Overall survival, % (95% CI)				.472
1 year	71.7 (64.0-78.1)	75.7 (65.2-83.4)	66.9 (54.9-76.5)	
2 year	66.2 (58.1-73.2)	65.4 (53.3-75.1)	65.6 (53.4-75.2)	
Relapse-free survival, % (95% CI)				.758
1 year	65.1 (57.2-71.9)	67.9 (56.9-76.6)	61.6 (49.5-71.7)	
2 year	59.1 (50.9-66.4)	58.4 (46.5-68.5)	58.8 (46.6-69.1)	
Non-relapse mortality, % (95% CI)				.967
100	5.0 (2.3-9.1)	6.8 (2.8-13.4)	2.7 (0.5-8.6)	
1 year	131 (8.4-18.9)	13.8 (7.5-22.0)	12.3 (6.0-21.0)	
2 year	15.4 (10.2-21.6)	17.4 (9.8-26.7)	13.7 (7.0-22.7)	
CIR, % (95% CI)				.712
1 year	21.8 (15.8-28.5)	18.3 (11.0-27.1)	26.0 (16.6-36.5)	
2 year	25.5 (18.9-32.6	24.2 (15.4-34.1	27.5 (17.8-38.1)	
GRFS, % (95% CI)				<.001
1 year	46.9 (39.0-54.4)	59.9 (48.8-69.3)	31.5 (21.3-42.2)	
2-year	37.0 (29.3-44.8)	50.2 (38.5-60.8)	21.8 (13.2-31.9)	

IQR indicates interquartile range.
* Outcome calculated among patients alive and without disease relapse.
† Causes of death were determined based on the CIBMTR criteria.

The cumulative incidence of grade 2-4 BK-virus-associated hemorrhagic cystitis (HC) was higher in patients receiving PTCY-TK (Day 180: 11.4% versus 2.7%, *P* = .038). Three patients had grade 3-4 BK HC, and 2 received PTCY-TK. Two of the 3 patients died with active HC disease caused by other complications (steroid-refractory aGVHD and a concomitant infection). The cumulative incidence of probable or proven invasive pulmonary aspergillosis was comparable between the 2 cohorts (Day 180 of 4.5% and 4.9%, *P* = .927). All patients were treated with triazoles, and 2 patients died of this complication.

GVHD

The cumulative incidences of grade II-IV and III-IV aGVHD at day 100 were 18.2% and 5.7% for patients receiving PTCY-TK, and 26.0% (*P* = .103) and 9.6% (*P* = .240) for those receiving other GVHD (Table 2, Figure 1). The estimated 2-year

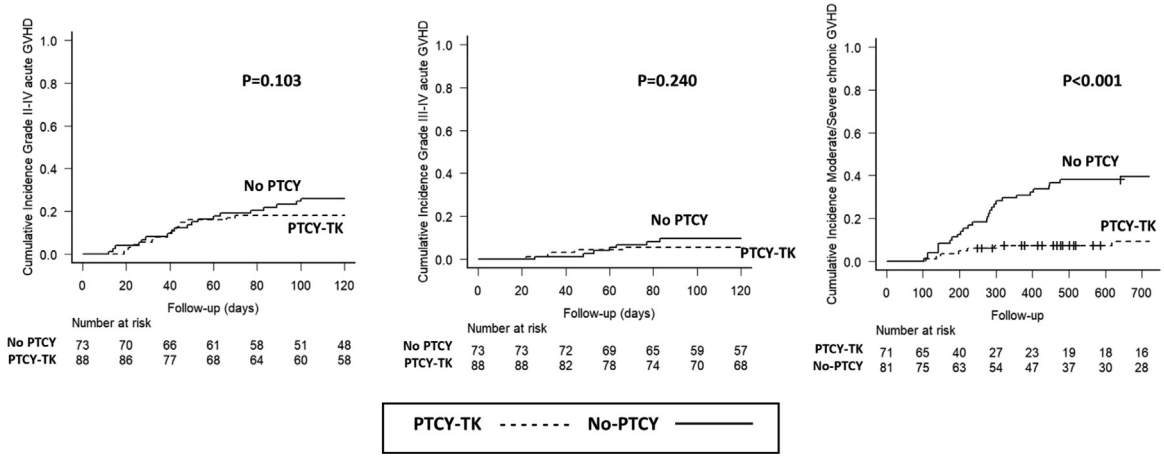

Figure 1. Cumulative incidences of acute and chronic GVHD.

cumulative incidence of any cGVHD and of moderate/severe cGVHD were 29.2% and 9.4% for patients receiving PTCY-TK, and 52.1% (P = .006) and 39.5% (P < .001) for those receiving other prophylaxis**.** Eleven (6.8%) patients died of steroid-refractory GVHD, either because of GVHD progression or concomitant infection, and only 1 (1.1%) patient received PTCY-TK for GVHD prevention.

Risk factors for GVHD are summarized in Supplementary Table S1 (univariate) and Table 3 (multivariate). The multivariate analysis showed that patients receiving PTCY-TK had a lower probability of being diagnosed with grade II-IV aGVHD (HR 0.32, P = .014), and the trend toward lower risk for presenting grade III-IV aGVHD was not statistically significant (HR 0.36, P = .11). In addition, the selection of 9/10 MMUD was found to increase the probability of being diagnosed with clinically relevant GVHD, independently of the selected GVHD prophylaxis.

As shown in Table 3, the use of PTCY-TK decreased the probability of being diagnosed with any grade of cGVHD (HR 0.43, P = .013), and more particularly the probability of moderate/severe cGVHD (HR 0.10, P < .001). Patients receiving other GVHD prophylaxis were 10 times more likely to be diagnosed with moderate/severe cGVHD than those receiving PTCY-TK. The multivariate regression model did not find any additional statistically significant risk factor for acute or chronic GVHD.

Survival outcomes

The use of PTCY-TK prophylaxis resulted in OS (2 years: 65.4% versus 65.6%, P = .472), RFS (2 years: 58.4% versus 58.8%, P = .758), NRM (2 years: 17.4% versus 13.7%, P = .967) and CIR (2-years: 24.2% versus 27.5%, P = .712) outcomes, comparable with the respective ones in the group of patients that received other GVHD prophylaxis (Table 2, Figure 2). However, GRFS rates were higher in patients receiving PTCY-TK than in patients with other prophylaxis (2-years: 50.2% versus 21.8%, P < .001). Disease relapse (14.8%) and infection (10.2%) were the most prevalent causes of death in the PTCY-TK group; for patients receiving other GVHD prophylaxis, the most prevalent causes of death were disease relapse (23.3%) and steroid-refractory GVHD (13.7%).

Table 4 summarizes the risk factors for OS, RFS, and GRFS**.** The main results provided by the multivariable analysis were that the use of PTCY-TK for GVHD prevention did not significantly affect OS (HR 0.74, P = .394) and RFS (HR 0.78, P = .441), but it had a statistically significant beneficial effect on GRFS (HR 0.42, P < .001). In addition, irrespective of the prophylaxis treatment, those patients with high and very high disease risk index had a higher risk of mortality than patients with low and intermediate-risk.

Within the subgroup of 91 (56.5%) patients known to be alive and without disease relapse, time to immunosuppression discontinuation was shorter in the PTCY-TK group than in the rest (7.8 versus 13.2 months, P < .001). At the last follow-up, 10 (10.7%) patients are still on immunosuppression because of moderate/severe cGVHD, and 8 of them did not receive PTCY-TK.

Main Results Among Patients Receiving PTCY-TK for GVHD Prophylaxis

Considering that PTCY-TK is the standard GVHD prophylaxis used at our institution when MSD and UD are selected, donor type related risk factors for GVHD, OS, and GRFS were further investigated in the cohort of 88 patients receiving PTCY-TK (Supplementary Table S3). Compared with alloHSCT performed using HLA-matched donors, the selection of MMUD resulted in a non-significant trend to higher rates of grade II-IV aGVHD (Day 100: 22.5% versus 14.6%; HR 1.30, P = .30) and III-IV (Day 100: 10.0% versus 2.1%, HR 5.01, P = .14), but rather increased the likelihood of being diagnosed with moderate/severe cGVHD (2 years: 18.9% versus 2.2%, HR 9.52, P = .049). In addition, a lower likelihood of moderate/severe cGVHD (2 years: 7.4% versus 17.3%, HR 0.58, 0.045) was documented when RIC regimens were used.

The selection of MMUD resulted in comparable rates of OS (HR 1.45, P = .333) and RFS (HR 1.43, P = .280), and of lower rates of GRFS (2 years: 65% versus 40%, HR 1.87, P = .043), compared with those obtained from HLA-matched donors alloHSCT. disease risk index was found to be a significant predictor for OS (high-very high versus low-intermediate disease risk: HR 4.21, P < .001) and GRFS (HR 2.67, P = .002).

DISCUSSION

This retrospective single-center study shows that PTCY-TK is a safer and a more effective drug combination for GVHD prevention among patients older than 50 years undergoing alloHSCT from MSD and UD than non-PTCY containing GVHD prevention strategies. PTCY-TK has improved GRFS rates and is considered one of the reasons of the progressive increment in the number of patients older than 65 years who underwent transplantation at our institution.

Table 3
Multivariate Analysis of Risk Factors for Acute and Chronic GVHD

Univariate	Risk factors for Grade II-IV acute GVHD		Risk factors for Grade III-IV acute GVHD		Risk factors for chronic GVHD		Risk factors for Moderate/severe chronic GVHD	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
GVHD prophylaxis PTCY-TK (versus other)	0.32 (0.13-0.79)	.014	0.36 (0.10-1.26)	.110	043 (0.22-0.86)	.018	0.10 (0.04-0.23)	.001
Intensity conditioning RIC (versus MAC)	1.39 (0.62-3.11)	.420	1.30 (0.34-4.92)	.690	0.91 (0.52-1.62)	.770	0.67 (0.33-4.74)	.270
Donor selection MMUD (versus MSD and MUD)	2.65 (1.12-6.24)	.025	4.26 (1.21-14.9)	.024	1.30 (0.65-2.60)	.460	1.84 (0.23-1.07)	.210
Donor age ≥ 50 (versus <50 years)	0.88 (0.40-1.90)	.750	2.14 (0.51-8.93)	.290	0.94 (0.53-1.69)	.860	0.50 (0.23-1.07)	.078
Range of Time 2018 – 2021 (versus 2014 – 2017)	1.18 (0.55-2.50)	.660	0.65 (0.18-2.28)	.510	0.73 (0.38-1.41)	.738	1.18 (0.51-2.73)	.690
Past history of aGVHD (any) Yes (versus not)	—	—	—	—	0.76 (0.43-1.33)	.340	0.63 (0.31-1.26)	.200

The multivariate analysis for the cumulative incidence of clinically relevant GVHD has been performed accounting relapse and death as competing events. Multivariate analysis for the cumulative incidence of cGVHD has been performed including adults that survived more than 100 days.

PTCY was first combined with TK and MMF (PTCY-TK-MMF) for haploHSCT at our institution, and progressively expanded to PB alloHSCT using other donor types. The use of MMF was interrupted out of the haploHSCT setting in 2016. This decision was based on the hypothesis that PTCY-TK would provide sufficient immunosuppression to permit engraftment and would minimize GVHD rates in T-cell replete PB alloHSCT from HLA matched and single-loci HLA mismatched donors [11,12]. Primary GF was only diagnosed in 2 patients, confirming the expectation that when MRD and UD donors are selected, PTCY-TK induces sufficient immunosuppression to permit a sustained engraftment after the stem cell infusion. A previous single-arm retrospective analysis, including 109 patients undergoing alloHSCT from UD and receiving PTCY-TK published by our group, demonstrated that the PTCY-TK prophylaxis was effective for GVHD prevention [12]. The present study shows that PTCY-TK prophylaxis is also safe and effective in older patients undergoing alloHSCT from MSD, MUD, and MMUD. And it shows that the rates of GVHD in MMUD alloHSCT were higher than in those performed from HLA-matched donors, especially when MAC regiments were administered. Remarkably, 40% of the patients older than 50 years that received grafts from MMUD and PTCY-TK prophylaxis were alive, without disease relapse, and without having had clinically relevant GVHD, at 2 years. These transplantation outcomes in MMUD alloHSCT are in line with those reported by other investigators using PTCY-containing GVHD prophylaxis [14–16]. Based on this evidence, PTCY-TK is the current GVHD prophylaxis at our institution when MMUD are selected, and research continues exploring whether the donor type impacts on the efficacy of PTCY-TK in elderly patients undergoing PB alloHSCT.

The multivariate analysis confirmed that PTCY-TK immunoprophylaxis for transplanted patients older than 50 years results in better GVHD prevention and in higher GRFS rates than conventional GVHD prophylaxis. This result is even more remarkable considering that the proportion of transplanted with MMUD donors was higher in the group of patients receiving PTCY-TK. Moreover, the effectiveness derived from the PTCT-TK prophylaxis has permitted a faster discontinuation of the immunosuppression of the treated patients. Rates of viral reactivations and infections were comparable between the 2 cohorts of patients with different GVHD prophylaxis, except for the higher risk of being diagnosed with BK-virus HC in the PTCY-TK group. However, despite of the increased proportion of HC documented in these patients, only 3 of them presented a clinically relevant episode that was successfully controlled with specific treatment. The proportion of patients with BSI was superior in the cohort of those receiving PTCY-TK. Higher risk for BSI has been reported in patients receiving PTCY-based GVHD prophylaxis, with incidences ranging between 30% and 50%, depending on the selected donor source [17–19]. Further analyses are required to determine the incidence and risk factors of BSI among patients receiving PTCY, in order to standardize the need of antimicrobial prophylaxis and improve supportive care.

Although other studies have documented the outcomes from PTCY-based GVHD prophylaxis in older patients [15,20-26], those that specifically explored the safety of this protocol in elderly candidates for alloHSCT are limited [27,28]. These studies find similar survival rates in transplanted patients with and without PTCY-TK prophylaxis, but in cohorts of patients with lower median age lower than that of the patients in our study [15,20-26]. Then our study confirms that PTCY-TK prophylaxis is also safe and effective in a cohort of patients with

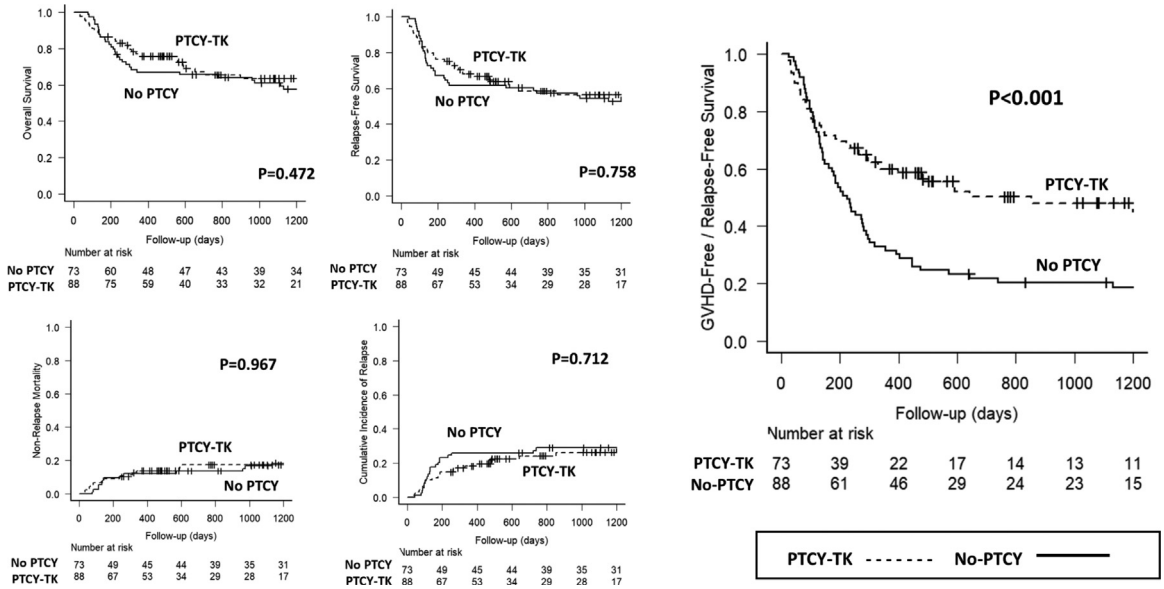

Figure 2. Main post-transplant outcomes according to the GVHD prophylaxis administered for alloHSCT.

50 years of age or older. Older patients have historically been excluded from intensive therapies such as alloHSCT, but this trend has changed over the last 2 decades because aging is heterogeneous and an erroneous summary of the patient's health status. Significantly, the effectiveness derived from PTCY-TK has improved GRFS, mainly because of the reduction in cGVHD rates, in older patients too, which explains the increasing trend in the number of patients older than 65 years undergoing alloHSCT at our institution.

The effectiveness of PTCY for GVHD prevention in HLA-matched related and UD alloHSCT is supported by different publications, although the conclusions are limited, either because the sample size is small or because the patients received a heterogeneous drug combination, including PTCY [15,20-26]. A recent prospective analysis demonstrated that PTCy combined with cyclosporine successfully reduced severe GVHD and improved GRFS rates, compared to the combination of CsA and MMF, after non-myeloablative matched related and unrelated PB alloHSCT [29]. Another prospective study also reports that the use of PTCY-TK-MMF resulted in superior GRFS rates than TK-MTX, secondary to a reduction of grade II-IV aGVHD, in adults undergoing RIC alloHSCT [26]. PTCY-based GVHD prophylaxis appeared to be more effective for aGVHD prevention than CsA-MTX for MSD alloHSCT, but the reported

Table 4
Risk Factors for OS, RFS, and GRFS

	Overall Survival		Relapse-Free Survival		GVHD-Free / Relapse-Free Survival	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Univariate Cox regression analysis						
GVHD prophylaxis PTCY-TK (versus other)	0.82 (0.49-1.38)	.472	0.92 (0.57-1.48)	.757	0.49 (0.33-0.73)	<.001
Age						
Continuous	1.02 (0.98- 1.07)	.253	1.02 (0.98-1.06)	.269	1.01 (0.97-1.03)	.823
≥65 (versus < 65)	1.10 (0.61-1.98)	.742	1.23 (0.72-2.08)	.440	0.97 (0.61.1.54)	.929
HCT-CI score >3 (versus 0-3)	1.12 (0.63-2.01)	.689	1.18 (0.69-2.01)	.527	0.90 (0.57-1.42)	.680
Karnofsky performance status 80-60% (versus 100-90%)	1.53 (0.86-2.73)	.145	1.37 (0.80-2.34)	.248	1.19 (0.75-1.89)	.438
Disease risk index: high – very high (Low – Int)	2.02 (1.19-3.42)	.008	1.73 (1.07-2.81)	.024	1.86 (1.24-2.80)	.002
Intensity conditioning: RIC (versus MAC)	1.24 (0.68-2.27)	.479	1.24 (0.72-2.12)	.424	1.02 (0.66-1.57)	.907
Donor selection						
MSD (versus MMUD)	0.83 (0.42-1.64)	.610	0.93 (0.51-1.76)	.834	1.29 (0.78-2.13)	.317
MUD (versus MMUD)	0.73 (0.39-1.37)	.336	0.70 (0.39-1.25)	.232	0.78 (0.48-1.27)	.329
Range of time: 2018 – 2021 (versus 2014 – 2017)	0.98 (0.58- 1.67)	.962	1.13 (0.69-1.82)	0.616	0.87 (0.58-1.30)	.508
Multivariate Cox Regression Analysis						
GVHD prophylaxis: PTCY-TK (versus other)	0.74 (0.37-1.46)	.394	0.78 (0.42-1.45)	.441	0.42 (0.25-0.70)	<.001
Age at transplantation ≥65 (versus < 65)	1.05 (0.57-1.94)	.853	1.16 (0.67-2.01)	.579	0.96 (0.60-1.54)	.879
HCT-CI score: >3 (versus 0-3)	1.32 (0.71-2.47)	.367	1.37 (0.79-2.40)	.257	0.90 (0.55-1.46)	.684
Karnofsky performance status 80%-60% (versus 100%-90%)	1.60 (0.88-2.88)	.116	1.36 (0.79-2.35)	.258	1.10 (0.69-1.75)	.686
Disease risk index: high – very high (Low – Int)	1.94 (1.12-3.34)	.016	1.65 (1.01-2.71)	.046	1.76 (1.15-2.67)	.007
Donor selection MMUD (versus MSD and MUD)	1.54 (0.77-3.06)	.218	1.42 (0.76-2.64)	.261	1.42 (0.84-2.39)	.185
Range of time 2018 – 2021 (versus 2014 – 2017)	1.08 (0.58-1.98)	.804	1.20 (0.69-2.09)	.498	1.23 (0.78-1.93)	.369

impact on disease relapse was not consistent [21,25]. In addition, PTCY combined with SIR and MMF provided better survival outcomes than those observed in patients receiving T-cell depleted PBSC grafts from HLA-matched donors, but resulted in higher rates of cGVHD [22]. The Acute Leukemia Working Party of the EBMT retrospectively compared the efficacy of PTCY-containing prophylaxis with ATG-based prophylaxis in a large cohort of adults with acute myeloid leukemia undergoing alloHSCT [15,23,24]. In MSD alloHSCT, cGVHD rates were lower in the ATG group [23]. On the other hand, in the 10/10 HLA-MUD setting, the use of PTCY provided GVHD rates and outcomes comparable to those obtained with ATG [24]. In the 9/10 HLA-MMUD setting, lower incidence of severe aGVHD and higher RFS and GRFS rates were observed using PTCY [15]. None of our patients received ATG for GVHD prevention, so the authors cannot comment on whether PTCY-TK would have provided results comparable to those obtained with GVHD prophylaxis containing ATG.

Other recent publications also report that PTCY-TK-MMF [26,30], PTCY-SIR-MMF [8,31], or dual T-cell depletion using PTCY, ATG, and CsA (PTCY-ATG-CsA) could be appropriate combinations when MSD and UD are selected [32]. PTCY combined with bortezomib in MRD and with bortezomib and ATG in MUD RIC PB alloHSCT has proved to be effective in the prevention of acute and chronic GVHD [33,34]. Our study provides evidence on transplantation outcomes from using a different clinical protocol for GVHD prevention and shows that PTCY-TK can be safety used in a more aging process patient population.

The retrospective nature of the study, together with the fact that the adoption of PTCY-TK as the institutional GVHD prophylaxis at our institution has been progressive over time, can be viewed as limitations of this research. Aware of the latter limitation, a time-defined variable was incorporated among the explanatory ones in the multivariate analysis to control for possible effects on transplant outcomes from changes in the management of the prophylaxis and from the experience acquired by the medical team over time. Results about immune reconstitution are not reported because data on this variable were not collected for this study.

PTCY-TK is today the standard GVHD prophylaxis for MSD, and UD (10/10 MUD and 9/10 MMUD) PB alloHSCT at our institution. The present comparative study reports that this GVHD prophylaxis provides superior GVHD control than conventional prophylaxis in adults aged 50 and older undergoing alloHSCT, with comparable relapse rates and overall mortality.

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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.2022.05.009.

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