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RESEARCH ARTICLE

Comparable relapse incidence after unrelated allogeneic stem cell transplantation with post-transplant cyclophosphamide versus conventional anti-graft versus host disease prophylaxis in patients with acute myeloid leukemia: A study on behalf of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation

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Abstract

We compared relapse incidence (RI) post-unrelated transplantation with post-transplant cyclophosphamide (PTCy) versus no PTCy graft-versus-host disease (GVHD) prophylaxis, in 7049 acute myeloid leukemia (AML) patients in remission, 707 with PTCy, and 6342 without (No PTCy). The patients in the PTCy group were younger, 52.7 versus 56.6 years ($p < .001$). There were more 9/10 donors in the PTCy group, 33.8% versus 16.4% ($p < .001$), and more received myeloablative conditioning, 61.7% versus 50.2% ($p < .001$). In the No PTCy group, 87.7% of patients received in vivo T-cell depletion. Neutrophil and platelet engraftment were lower in the PTCy versus No PTCy group, 93.8% and 80.9% versus 97.6% and 92.6% ($p < .001$). RI was not significantly different in the PTCy versus the No PTCy group, hazard ratio (HR) of 1.11 (95% confidence interval [CI] 0.9–1.37) ($p = .31$). Acute GVHD grades II–IV and III–IV, were significantly lower in the PTCy versus the No PTCy group, HR of 0.74 (95% CI 0.59–0.92, $p = .007$) and HR = 0.56 (95% CI 0.38–0.83, $p = .004$), as were total and extensive chronic GVHD, HRs of 0.5 (95% CI 0.41–0.62, $p < .001$) and HR = 0.31 (95% CI 0.22–0.42, $p < .001$). Non-relapse mortality (NRM) was significantly lower with PTCy versus the No PTCy group, HR of 0.67 (95% CI 0.5–0.91, $p = .007$). GVHD-free, relapse-free survival (GRFS) was higher in the PTCy versus the No PTCy group, HR of 0.69 (95% CI 0.59–0.81, $p = .001$). Leukemia-free survival (LFS) and overall survival (OS) did not differ between

Data presented in part at the ASH 2023 meeting, San Diego (abstract 237).

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the groups. In summary, we observed comparable RI, OS, and LFS, significantly lower incidences of GVHD and NRM, and significantly higher GRFS in AML patients undergoing unrelated donor-hematopoietic stem cell transplantation with PTCy versus No PTCy GVHD prophylaxis.

1 | INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the treatment of choice for high-risk acute myeloid leukemia (AML).[1,2] Relapse is the leading cause of treatment failure after Allo-HSCT for AML reaching up to 50% depending on the leukemic risk, intensity of the preparative regimen, and disease status at Allo-HSCT.[3,4] The anti-leukemic effect mediated by the Allo-HSCT is based on the chemotherapy of the pre-transplantation conditioning but, moreover, on the so-called graft-versus-leukemia (GVL) effect in which donor immunocompetent cells in the graft recognize antigens on the residual leukemic cells as foreign and initiate immune-mediated clearance of residual leukemic cells in the host.[5–8] Donor histocompatibility is one of the factors affecting the allogeneic GVL effects and in the unrelated setting, there is a strong correlation between chronic cGVHD and the GVL effect post both matched and mismatched-unrelated donor HSCT (UD-HSCT).[7–10] However, these data were generated in UD-HSCT in which a calcineurin inhibitor (CNI) with or without in vivo T cell depletion by anti-thymocyte globulin (ATG) was the cornerstone for GVHD prophylaxis.[11–13] Post-transplant cyclophosphamide (PTCy)-based anti-GVHD prophylaxis which was pioneered in the haploidentical setting[14,15] has proven to be highly effective in preventing GVHD and reducing rates of both cGVHD and non-relapse mortality (NRM) inducing better immune tolerance and immune reconstitution.[16,17] PTCy given early after human leukocyte antigen (HLA)-mismatched graft infusion, selectively inhibits rapidly proliferating alloreactive T cells in both the graft-versus-host and host-versus-graft direction, while preserving slowly dividing memory and regulatory T cells (Tregs) in the graft, upregulating the latter leading to long-term immune tolerance and GVHD control.[18–20] Given these unprecedented results, PTCy is increasingly being used as GVHD prophylaxis post-UD-HSCT for patients with AML.[21–24] However, due to the PTCy inducing long immune tolerance, down-regulation of alloreactive T cells as well as donor-derived natural killer (NK) cells and upregulation of Tregs, and as PTCy reduces cGVHD that correlates with the GVL effect in the unrelated setting, there is, at least from a theoretical point of view, a concern that in the unrelated setting PTCy-based anti-GVHD prophylaxis will be associated with increased post-transplantation relapse rate. Although in the haploidentical setting, PTCy was not shown to increase leukemic relapse post-transplant when data were analyzed according to the disease risk index (DRI) the situation may differ in the unrelated setting.[25] Therefore, whether PTCy-based anti-GVHD prophylaxis will be associated with an increased relapse incidence (RI) in the unrelated setting is largely unknown. We thus compared the RI post-9–10/10 HLA UD-HSCT with PTCy versus conventional anti-GVHD prophylaxis in patients with AML using the registry data of the Acute Leukemia Working Party (ALWP) of the European Society for Blood and Marrow Transplantation (EBMT).

2 | SUBJECTS AND METHODS

2.1 | Study design and data collection

This was a retrospective, multicenter study. Data were provided by the registry of the ALWP of the EBMT. The EBMT is a non-profit scientific society representing more than 600 transplant centers, mainly located in Europe, which are required to report all consecutive stem cell transplantations and follow-ups once a year. Data are entered, managed, and maintained in a central database. Since January 1, 2003, all transplantation centers have been required to obtain written informed consent before data registration with the EBMT. Data accuracy is assured by the individual transplant centers and by quality control measures that include verification of the computer print-out of the entered data, cross-checking with the national registries, on-site visits to selected teams, and regular internal and external audits. The study was approved by the ALWP of the EBMT institutional review board and conducted per the Declaration of Helsinki and Good Clinical Practice guidelines. The results of disease assessments at HSCT were also submitted and form the basis of this report.

2.2 | Criteria for selection

Eligibility criteria for this analysis included adult patients ≥18 years of age with de novo AML who underwent their first Allo-HSCT from a 9–10/10 HLA-matched UD in CR1 between 2014 and 2021. Only peripheral blood stem cells (PBSC) as a graft source were allowed. Eligible GVHD prophylaxis included PTCy or non-PTCy-based (in vivo T-cell depletion and or calcineurin inhibitor) (No PTCy). ATG was allowed (Table 1). The exclusion criteria were allo-HSCT from siblings, haploidentical or cord blood donors; previous history of HSCT, disease status >CR1 or active disease, bone marrow (BM) grafts, and T cell-depleted HSCTs. Pre-transplantation preparative regimens included both reduced-intensity conditioning (RIC) and myeloablative conditioning (MAC).

Data collected included recipient and donor characteristics (age, gender, and cytomegalovirus [CMV] serostatus), Karnofsky performance status (KPS), disease characteristics including type, disease status, cytogenetic risk (European LeukemiaNet [ELN] 2017 cytogenetics classification), pre-HSCT molecular remission defined by measurable residual disease (MRD), year of transplant, type of

conditioning regimen, and GVHD prophylaxis, based on the reports from individual transplant centers as per previously established criteria.[26] The conditioning regimen was defined as MAC when containing total body irradiation (TBI) with a dose >6 Gray or a total dose of busulfan (Bu) >8 mg/kg or >6.4 mg/kg when administered orally or intravenously, respectively. All other regimens were defined as RIC.[26] Grading of acute GVHD (aGVHD) was performed using established criteria.[27] cGVHD was classified as limited or extensive according to published criteria.[28] For this study, all necessary data were collected according to the EBMT guidelines, using the EBMT minimum essential data forms. The list of institutions contributing data to this study is provided in the Supplemental Appendix.

2.3 | Statistical analysis

Patient, disease, and transplant-related characteristics for the two cohorts were compared using the median, interquartile range (IQR), and range for quantitative variables, and frequency and percentage for categorical variables. Association between the variables was tested with the Mann–Whitney *U* test for numerical variables, and the χ^2 or Fisher's exact test for categorical variables.

The primary study endpoint was the incidence of relapse, the secondary endpoints included the incidence of neutrophil and platelet engraftment, the incidence of aGVHD and cGVHD, overall survival (OS) leukemia-free survival (LFS), NRM, and GVHD-free, relapse-free survival (GRFS). All endpoints were measured from the time of transplantation. Myeloid engraftment was defined as achieving an absolute neutrophil count (ANC) of 0.5×10^9/L for three consecutive days. Platelet engraftment was defined as achieving a platelet count of 20×10^9/L for three consecutive days. OS was defined as time to death from any cause. LFS was defined as survival with no evidence of relapse or progression. NRM was defined as death from any cause without previous relapse or progression. We used modified GRFS criteria. GRFS events were defined as the first event among grades III–IV aGVHD, extensive cGVHD, relapse, or death from any other cause.[29]

Median follow-up was calculated by the reverse Kaplan–Meier method. All outcomes were censored at 2 years post-transplantation to allow for the difference in follow-up periods between groups. The probabilities of OS, LFS, and GRFS were calculated using the Kaplan–Meier method. aGVHD, cGVHD, RI, and NRM were estimated using cumulative incidence curves in a competing risk setting. The RI and NRM were calculated using cumulative incidence functions in a competing risk setting, with death in remission being treated as a competing event for relapse. Death was considered a competing event for engraftment. To estimate the cumulative incidence of acute or cGVHD, relapse, and death were considered as competing events. Univariate analyses were performed using the log-rank test for LFS, OS, and GRFS whereas Gray's test was used to compare cumulative incidence estimates. RI and NRM were mutually competing events. Multivariate analyses were performed using the Cox proportional-hazards regression model.[30] Known risk factors and unbalanced variables between groups were included in the model. To take into account any heterogeneity between centers, we introduced a random effect (or frailty) into the Cox multivariate models.[31] In order to evaluate the robustness of the results, a pair-matched analysis has been performed matching 1 patient without PTCy for 1 patient with PTCy. It has been realized using a propensity score with a caliper fixed at 0.5 and including all the variables of the multivariate analysis. Results were expressed as the hazard ratio (HR) with a 95% confidence interval (CI). All *p* values were two-sided with a type 1 error rate fixed at .05. Statistical analyses were performed with SR 4.0.2 (R Core Team).[32]

3 | RESULTS

In total, 7049 patients met the inclusion criteria, 707 (10%) received PTCy, and 6342 received CNI-based (No PTCy) GVHD prophylaxis. Median follow-up was 1.9 (range 1.7–2.1) and 2.9 (range 2.8–3.0) years, respectively ($p < .001$). Table 1 shows the baseline demographic and clinical characteristics. The patients in the PTCy group were younger, median age was 52.7 (range 18–77.5) versus 56.6 (range 18–80.3) years ($p < .001$), while gender did not differ (56.4% and 53.6% of the patients were male, respectively). The median year of the transplant was 2019 (range 2014–2021) and 2018 (2014–2021), respectively ($p < .0001$). There were more HLA 9/10 UDs in the PTCy compared to the No PTCy group; 33.8% versus 16.4%, respectively ($p < .001$). Cytogenetic risk category did not differ between the groups, ($p = .54$) (Table 1). In total, 301 (5.1%) patients were categorized as having a favorable cytogenetic risk, while intermediate and adverse risk were represented by 3975 (67.9%) and 1577 (26.9%) patients, respectively (data was missing for 1196 [17%] patients). The KPS score did not differ between the groups and was ≥90 in 76.9% versus 78.8% ($p = .27$), respectively. The PTCy group was characterized by a longer period from diagnosis to Allo-HSCT being 5.3 (range 1.6–28.8) versus 5.1 (range 0–30) months, ($p = .004$), respectively, a higher frequency of female donor to male recipient combination (15.2% vs. 10.9%, $p = .001$) and higher frequency of patients who were seropositive for CMV when compared with the No PTCy group (74.6% vs. 65.5%, $p < .0001$). More patients in the PTCy versus No PTCy group received MAC, 61.7% versus 50.2%, ($p < .001$), and TBI 17.8% versus 13.3% ($p = .001$), respectively. The most frequent conditioning regimen for both groups was Bu/fludarabine (Flu) in 59.8% and 45.8%, followed by Bu/Cy in 5.3% and 6.4% of patients in the PTCy and No PTCy groups, respectively. In the No PTCy group, 87.7% of the patients received in vivo T-cell depletion, and the most frequent anti-GVHD prophylaxis was cyclosporine A (CSA) in combination with methotrexate in 46.4% and CSA plus mycophenolate mofetil in 30.4% of the patients (Table 1).

3.1 | Transplantation outcome

Day 30 neutrophil engraftment was 93.8% in the PTCy compared to 97.6% in the No PTCy group (Table 2, Figure 1A). Platelet engraftment was 80.9% in the PTCy compared to 92.6% in the No PTCy group (Table 2, Figure 1A). The incidence of aGVHD grades II–IV and III–IV at 100 days were 23.9% (95% CI 20.7%–27.1%) and 6.6% (95% CI 4.9%–8.6%) in patients receiving PTCy compared with 25.2%

TABLE 1 Patient and transplant characteristics.

	Total 7049	No PTCy 6342	PTCy 707	*p* Value
Age patient (years)				
Median (range) [IQR]	56.34 (18–80.3) [45.3–64]	56.6 (18–80.3) [45.8–64.2]	52.7 (18–77.5) [40.7–62.5]	<.0001
Year HSCT				
Median (range) [IQR]	2018 (2014–2021) [2016–2020]	2018 (2014–2021) [2016–2020]	2019 (2014–2021) [2017–2020]	<.0001
Time from diagnosis to HSCT (months)				
Median (range) [IQR]	5.16 (0–30) [4.1–6.6]	5.1 (0–30) [4.1–6.6]	5.3 (1.6–28.8) [4.2–7.3]	.004
Missing	38	35	3	
Sex patient				
Female	3250 (46.1)	2942 (46.4)	308 (43.6)	.16
Male	3793 (53.9)	3395 (53.6)	398 (56.4)	
Missing	6	5	1	
Sex donor				
Female	1980 (28.3)	1770 (28.1)	210 (29.8)	.35
Male	5012 (71.7)	4518 (71.9)	494 (7.2)	
Missing	57	54	3	
Female to male donor				
No	6219 (88.7)	5622 (89.1)	597 (84.8)	.001
Yes	795 (11.3)	688 (10.9)	107 (15.2)	
Missing	35	32	3	
CMV patient				
Negative	2339 (33.5)	2161 (34.4)	178 (25.4)	<.0001
Positive	4652 (66.5)	4130 (65.6)	522 (74.6)	
Missing	58	51	7	
CMV donor				
Negative	3664 (52.6)	3296 (52.6)	368 (52.5)	.96
Positive	3305 (47.4)	2972 (47.4)	333 (47.5)	
Missing	80	74	6	
Karnofsky score				
<90	1538 (22.9)	1393 (23.1)	145 (21.2)	.27
≥90	5173 (77.1)	4635 (76.9)	538 (78.8)	
Missing	338	314	24	
Type of donor				
UD 10/10	5768 (81.8)	5300 (83.6)	468 (66.2)	<.0001
UD 9/10	1281 (18.2)	1042 (16.4)	239 (33.8)	
Cytogenetic risk				
Favorable	301 (5.1)	267 (5.1)	34 (5.6)	.54
Intermediate	3975 (67.9)	3558 (67.8)	417 (69.2)	
Adverse	1577 (26.9)	1425 (27.1)	152 (25.2)	
Missing	1196	1092	104	
TBI				
No	6079 (86.2)	5498 (86.7)	581 (82.2)	.001
Yes	970 (13.8)	844 (13.3)	126 (17.8)	
Myeloablative regimen				
No	3428 (48.6)	3157 (49.8)	271 (38.3)	<.001
Yes	3621 (51.4)	3185 (50.2)	436 (61.7)	

(Continues)

TABLE 1 (Continued)

	Total 7049	No PTCy 6342	PTCy 707	*p* Value
In vivo T cell depletion				
No	1378 (19.5)	782 (12.3)	596 (84.3)	<.0001
Yes	5671 (80.5)	5560 (87.7)	111 (15.7)	
Molecular remission				
No	1200 (35.2)	1081 (35.1)	119 (36.3)	.66
Yes	2211 (64.8)	2002 (64.9)	209 (63.7)	
Missing	3638	3259	379	
Conditioning regimen				
BuCy based	1015 (14.4)	970 (15.3)	45 (6.4)	Not done
BuFlu based	3326 (47.2)	2903 (45.8)	423 (59.8)	
FluCy/FluMel based	851 (12.1)	819 (12.9)	32 (4.5)	
TBI based	970 (13.8)	844 (13.3)	126 (17.8)	
Treo based	887 (12.6)	806 (12.7)	81 (11.5)	
GVHD prophylaxis				
CSA based	886 (12.6)	779 (12.3)	107 (15.1)	Not done
CSA MMF/MTX or CSA MTX based	2960 (42)	2943 (46.4)	17 (2.4)	
CSA/MMF based	2120 (30.1)	1928 (30.4)	192 (27.2)	
MMF based	582 (8.3)	298 (4.7)	284 (40.2)	
MMF/MTX or MTX based	266 (3.8)	249 (3.9)	17 (2.4)	
SIRO or TACRO	235 (3.3)	145 (2.3)	90 (12.7)	

Note: Unless otherwise stated, results expressed as frequency (%).
Abbreviations: Bu, busulfan; CMV, cytomegalovirus; CSA, cyclosporine A; Cy, cytoxan; Flu, fludarabine; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; IQR, interquartile range; Mel, melphalan; MMF, mycophenolate mofetil; MTX, methotrexate; PTCy, post-transplant cyclophosphamide; SIRO, sirolimus; TACRO, tacrolimus; TBI, total body irradiation; Treo, treosulfan; UD, unrelated donor.

TABLE 2 Transplantation outcomes according to graft-versus-host disease (GVHD) prophylaxis regimen.

Outcomes	Total Estimation (95% CI)	No PTCy Estimation (95% CI)	PTCy Estimation (95% CI)
Median FU (years)	2.8 (2.6–2.9)	2.9 (2.8–3)	1.9 (1.7–2.1)
OS (2 years)	66.3 (65.1–67.5)	65.9 (64.6–67.2)	70.3 (66.2–74)
LFS (2 years)	58.5 (57.2–59.7)	57.9 (56.6–59.2)	64.5 (60.4–68.4)
RI (2 years)	26.7 (25.6–27.8)	26.9 (25.7–28.1)	24.9 (21.3–28.6)
NRM (2 years)	14.8 (13.9–15.7)	15.2 (14.3–16.2)	10.6 (8.2–13.2)
Poly recovery (30 days)	97.2 (96.8–97.5)	97.6 (97.2–97.9)	93.8 (91.7–95.3)
Poly recovery (60 days)	98.7 (98.4–98.9)	98.7 (98.4–99)	98.3 (96.9–99)
Platelet recovery (≥20) (30 days)	91.4 (90.7–92)	92.6 (91.9–93.2)	80.9 (77.6–83.7)
Platelet recovery (≥20) (60 days)	95.8 (95.3–96.2)	96.2 (95.7–96.7)	92 (89.7–93.9)
aGVH-II/IV (100 days)	25.1 (24–26.1)	25.2 (24.1–26.3)	23.9 (20.7–27.1)
aGVH-III/IV (100 days)	8.2 (7.6–8.9)	8.4 (7.7–9.1)	6.6 (4.9–8.6)
GRFS (2 years)	46.6 (45.3–47.9)	45.9 (44.6–47.3)	53.3 (49–57.3)
cGVHD (2 years)	32.4 (31.2–33.6)	32.7 (31.4–33.9)	29.2 (25.5–33.1)
cGVHD Ext (2 years)	13.4 (12.5–14.3)	13.6 (12.6–14.5)	12 (9.4–15)

Note: Unless otherwise stated, results expressed as frequency.
Abbreviations: aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CI, confidence interval; Ext, extensive; GRFS, GVHD-free, relapse-free survival; LFS, leukemia-free survival; NRM, non-relapse mortality; OS, overall survival; Poly, polymorphonuclear leukocytes; PTCy, post-transplant cyclophosphamide; RI, relapse incidence.

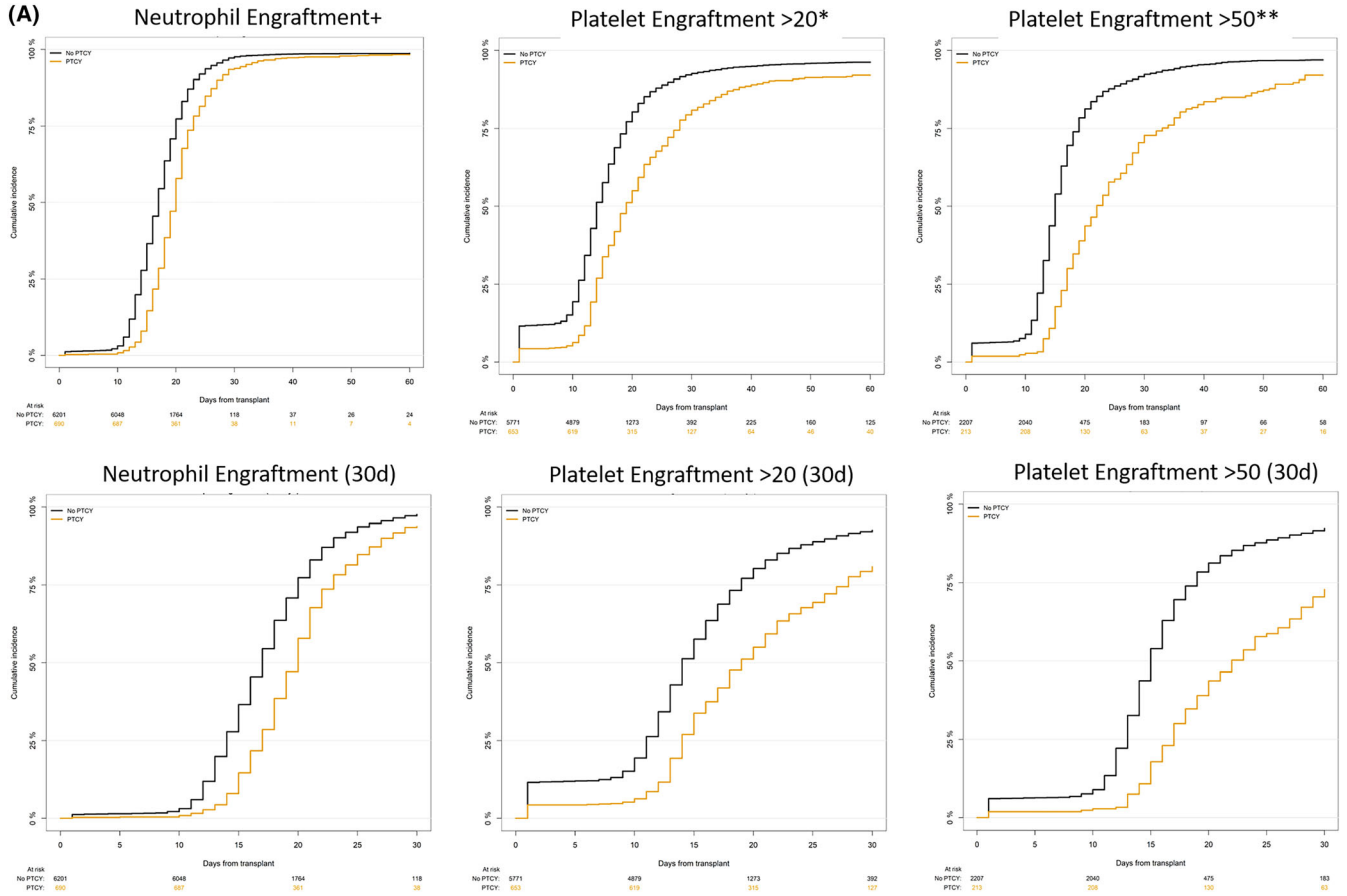

FIGURE 1 Outcomes of unrelated allogeneic stem cell transplantation with post-transplant cyclophosphamide (PTCy) compared with No PTCy (in vivo T-cell depletion or calcineurin inhibitor-based graft-versus-host disease [GVHD] prophylaxis) in patients with acute myeloid leukemia: (A) Neutrophil (absolute neutrophil count [ANC] ≥ 0.5 × 10^9/L) and platelet (≥20 × 10^9/L; ≥50 × 10^9/L) engraftment ($n = 2426$); (B) acute (a) GVHD II–IV and III–IV and chronic (c) GVHD total and extensive (C) Transplantation outcome—relapse incidence (RI), non-relapse mortality (NRM), leukemia-free survival (LFS), overall survival (OS), GVHD-free, relapse-free survival (GRFS). [Color figure can be viewed at wileyonlinelibrary.com]

(95% CI 24.1%–26.3%) and 8.4% (95% CI 7.7%–9.1%) in the patients with No PTCy GVHD prophylaxis, respectively (Table 2, Figure 1B). The 2-year incidence of total and extensive cGVHD were 29.2% (95% CI 25.5%–33.1%) and 12% (95% CI 9.4%–15%) in the PTCy compared to 32.7% (95% CI 31.4%–33.9%) and 13.6% (95% CI 12.6%–14.5%) in the No PTCy group, respectively (Table 2, Figure 1B). The 2-year RI was 24.9% (95% CI 21.3%–28.6%) versus 26.9% (95% CI 25.7%–28.1%) in patients receiving PTCy and those who received conventional anti-GVHD prophylaxis, respectively (Table 2, Figure 1B). Two-year NRM was 24.9% (95% CI 21.3%–28.6%) and GRFS 53.3% (95% CI 49%–57.3%) in the PTCy versus 26.9% (95% CI 25.7%–28.1%) and 45.9% (95% CI 44.6%–47.3%) in the No PTCy groups, respectively (Table 2, Figure 1B). Two-year LFS and OS were 64.5% (95% CI 60.4%–68.4%) versus 57.9% (95% CI 56.6%–59.2%) and 70.3% (95% CI 66.2%–74%) versus 65.9% (95% CI 64.6%–67.2%), respectively (Table 2, Figure 1C).

3.2 | Multivariate analysis

In the multivariate analysis, RI did not differ between patients receiving PTCy compared to those that did not receive PTCy-based GVHD prophylaxis, HR = 1.11 (95% CI 0.9–1.37, $p = .31$) (Table 3). Day 30 neutrophil and platelet engraftment was lower in the PTCy compared to the No PTCy group, HR = 1.67 (95% CI 1.47–1.92, $p < .001$) and HR = 2.44 (95% CI 2.17–2.78, $p < .001$), respectively (Table 3). The incidence of aGVHD II–IV, aGVHD III–IV, cGVHD all grades, and extensive cGVHD was lower in the PTCy compared to the No PTCy group: HR = 0.74 (95% CI 0.59–0.92, $p = .007$), HR = 0.56 (95% CI 0.38–0.83, $p = .004$), HR = 0.5 (95% CI 0.41–0.62, $p < .001$) and HR = 0.31 (95% CI 0.22–0.42, $p < .001$), respectively (Table 3). NRM was significantly lower with PTCy compared to the No PTCy group with an HR of 0.69 (95% CI 0.5–0.91, $p = .01$). GRFS was higher in the PTCy versus the No PTCy group, with an HR = 0.69 (95% CI 0.59–0.81, $p < .01$). LFS and OS did not differ significantly between the groups, with HRs of 0.93 (95% CI 0.78–1.1, $p = .39$) and HR = 0.93 (95% CI 0.77–1.12, $p = .45$), respectively (Table 3). Risk factors for RI were adverse cytogenetics and female donor to male patient transplant combination. 9/10 versus 10/10 HLA matching was a prognostic factor for increased risk of aGVHD, cGVHD, NRM, GRFS, LFS, and OS. Older patient age (per 10 years) was a prognostic factor for NRM, LFS, OS, GRFS, and grades II–IV aGVHD. CMV seropositivity in patients was a prognostic factor for increased hazard of NRM,

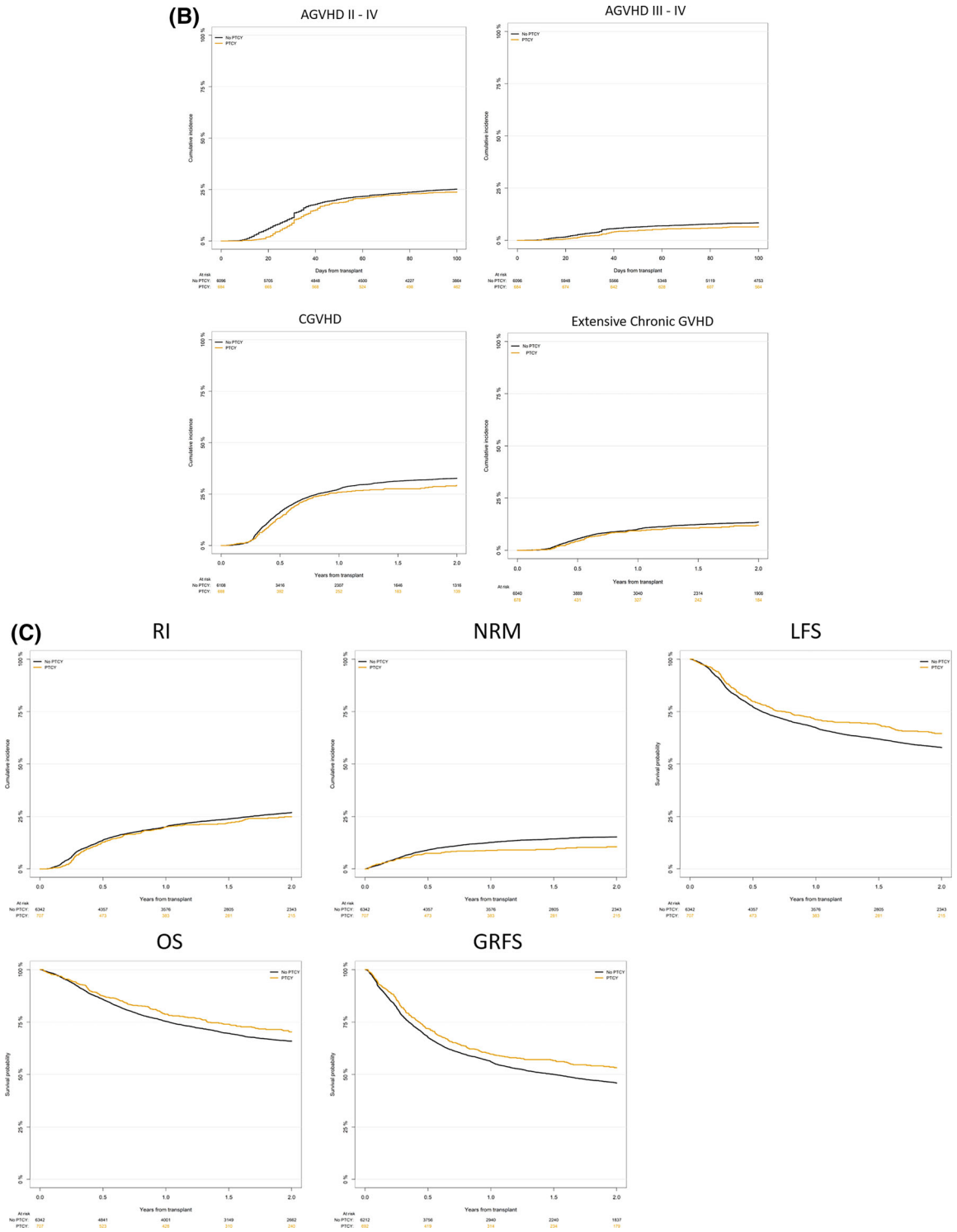

FIGURE 1 (Continued)

LFS, OS, GRFS, and cGVHD and extensive cGVHD. Cytogenetic risk was also a prognostic factor for LFS, OS, and GRFS. A female donor to male patient transplant combination was a prognostic factor for NRM, aGVHD grades II–IV, cGVHD and extensive cGVHD. Year of HSCT was a risk factor for aGVHD all grades, OS and GRFS. In vivo T-cell depletion was a prognostic factor for aGVHD grades II–IV, cGVHD, extensive cGVHD and GRFS. Finally, TBI was a prognostic factor for platelet engraftment and 9/10 versus 10/10 HLA matching, year of HSCT, a female donor to male patient transplant combination, MAC and in vivo T-cell depletion were prognostic factors for platelet engraftment (Table 3). Using the pair-match analysis, the results of the multivariate analyses were confirmed, RI: HR 1.04, $p = .75$;

TABLE 3 Multivariate analysis.

	LFS		OS		RI		NRM	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
PTCy								
No PTCy	1		1		1		1	
PTCy	0.93 (0.78–1.1)	.39	0.93 (0.77–1.12)	.45	1.11 (0.9–1.37)	.31	0.67 (0.5–0.91)	0.01
Year of HSCT by 5 years	0.94 (0.85–1.03)	.17	0.89 (0.8–0.99)	.03	0.98 (0.87–1.1)	.69	0.86 (0.73–1)	0.056
Age by 10 years	1.11 (1.07–1.15)	<.001	1.2 (1.15–1.24)	<.001	1 (0.96–1.04)	.86	1.39 (1.31–1.48)	<0.001
Donor type								
UD 10/10	1		1		1		1	
UD 9/10	1.23 (1.12–1.35)	<.001	1.31 (1.19–1.45)	<.001	1.03 (0.91–1.17)	.62	1.61 (1.39–1.87)	<0.001
Cytogenetics								
Favorable	1		1		1		1	
Intermediate	1.38 (1.1–1.74)	.005	1.43 (1.1–1.85)	.008	1.49 (1.11–2)	.008	1.2 (0.83–1.74)	0.32
Poor	2.33 (1.85–2.95)	<.001	2.51 (1.92–3.28)	<.001	2.86 (2.12–3.84)	<.001	1.58 (1.08–2.31)	0.02
Unknown	1.5 (1.18–1.92)	<.001	1.63 (1.23–2.15)	<.001	1.6 (1.17–2.18)	.003	1.36 (0.92–2.02)	0.12
Female donor to male recipient								
No	1		1		1		1	
Yes	1 (0.9–1.13)	.93	1.06 (0.94–1.21)	.33	0.83 (0.71–0.97)	.02	1.33 (1.12–1.59)	<0.001
CMV patients								
Negative	1		1		1		1	
Positive	1.14 (1.05–1.23)	.002	1.21 (1.11–1.33)	<.001	1.03 (0.93–1.14)	.58	1.37 (1.19–1.58)	<0.001
Myeloablative regimen								
No	1		1		1		1	
Yes	0.94 (0.86–1.03)	.2	0.99 (0.89–1.09)	.81	0.91 (0.81–1.02)	.1	1.02 (0.87–1.18)	0.83
TBI								
No	1		1		1		1	
Yes	1.04 (0.91–1.18)	.6	1.13 (0.98–1.31)	.09	0.94 (0.8–1.11)	.47	1.19 (0.95–1.49)	0.12
In vivo T cell depletion								
No	1		1		1		1	
Yes	1.11 (0.97–1.27)	.12	1.14 (0.98–1.32)	.09	1.17 (0.99–1.39)	.06	1.07 (0.86–1.33)	.55
	AGVH II–IV		AGVH III–IV		CGVH		GRFS	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
PTCy								
No PTCy	1		1		1		1	

(Continues)

TABLE 3 (Continued)

	AGVH II–IV		AGVH III–IV		CGVH		GRFS	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
PTCy	0.74 (0.59–0.92)	.007	0.56 (0.38–0.83)	.004	0.5 (0.41–0.62)	<.001	0.69 (0.59–0.81)	<.001
Year of HSCT by 5 years	0.8 (0.71–0.9)	<.001	0.73 (0.6–0.9)	.003	0.91 (0.81–1.02)	.1	0.91 (0.83–0.99)	.02
Age by 10 years	0.96 (0.92–1)	.03	0.92 (0.86–0.99)	.03	1.02 (0.98–1.06)	.32	1.04 (1.01–1.08)	.005
Donor type								
UD 10/10	1		1		1		1	
UD 9/10	1.38 (1.22–1.55)	<.001	1.56 (1.28–1.91)	<.001	1.21 (1.08–1.37)	<.001	1.27 (1.17–1.39)	<.001
Cytogenetics								
Favorable	1		1		1		1	
Intermediate	1.12 (0.87–1.45)	.38	1.38 (0.84–2.26)	.2	1.07 (0.85–1.35)	.58	1.29 (1.06–1.56)	.01
Adverse	1.31 (1–1.71)	.046	1.62 (0.97–2.7)	.06	1.14 (0.9–1.46)	.28	1.93 (1.58–2.35)	<.001
Unknown	1 (0.75–1.33)	1	1.25 (0.73–2.12)	.42	1.04 (0.8–1.34)	.79	1.37 (1.11–1.69)	.003
Female donor to male recipient								
No	1		1		1		1	
Yes	1.2 (1.04–1.39)	.01	1.14 (0.89–1.47)	.3	1.31 (1.14–1.5)	<.001	1.05 (0.94–1.16)	.38
CMV patients								
Negative	1		1		1		1	
Positive	1 (0.9–1.11)	.95	1.03 (0.86–1.24)	.74	1.2 (1.09–1.33)	<.001	1.14 (1.06–1.23)	<.001
Myeloablative regimen								
No	1		1		1		1	
Yes	0.89 (0.79–1.01)	.06	0.88 (0.72–1.08)	.23	0.98 (0.87–1.1)	.76	0.93 (0.85–1.01)	.08
TBI								
No	1		1		1		1	
Yes	1.04 (0.86–1.26)	.67	0.86 (0.64–1.17)	.34	1.15 (0.98–1.35)	.1	1.02 (0.91–1.15)	.69
In vivo T cell depletion								
No	1		1		1		1	
Yes	0.79 (0.67–0.95)	.01	0.8 (0.6–1.05)	.11	0.64 (0.54–0.75)	<.001	0.81 (0.72–0.91)	<.001

	CGVH ext		Neutrophil engraftment		Platelet engraftment	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
PTCy						
No PTCy	1		1		1	
PTCy	0.31 (0.22–0.42)	<.001	1.67 (1.47–1.92)	<.001	2.44 (2.17–2.78)	<.001
Year of HSCT by 5 years	1.02 (0.86–1.22)	.79	1.04 (0.98–1.1)	.19	1.15 (1.08–1.23)	.001

TABLE 3 (Continued)

	CGVH ext		Neutrophil engraftment		Platelet engraftment	
	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value	HR (95% CI)	*p* Value
Age by 10 years	1.02 (0.96–1.09)	.43	1.02 (1–1.05)	.03	0.99 (0.97–1.02)	.5
Donor type						
UD 10/10	1		1		1	
UD 9/10	1.32 (1.1–1.57)	.002	1.03 (0.96–1.1)	.44	1.15 (1.06–1.22)	<.001
Cytogenetics						
Favorable	1		1		1	
Intermediate	1.09 (0.76–1.55)	.64	0.96 (0.84–1.08)	.51	1.02 (0.89–1.16)	.77
Adverse	1.17 (0.81–1.7)	.4	0.96 (0.84–1.1)	.55	1.11 (0.97–1.28)	.13
Unknown	1.02 (0.69–1.51)	.92	0.98 (0.85–1.14)	.79	1.08 (0.94–1.27)	.25
Female donor to male recipient						
No	1		1		1	
Yes	1.34 (1.1–1.64)	.004	1.06 (0.99–1.16)	.1	1.12 (1.03–1.22)	.008
CMV patients						
Negative	1		1		1	
Positive	1.26 (1.08–1.46)	.003	0.97 (0.92–1.03)	.32	1.03 (0.97–1.1)	.26
Myeloablative regimen						
No	1		1		1	
Yes	1.01 (0.85–1.2)	.89	0.92 (0.86–0.98)	.01	1.15 (1.08–1.22)	<.001
TBI						
No	1		1		1	
Yes	1.21 (0.95–1.54)	.13	1.33 (1.20–1.47)	<.001	1.12 (1.02–1.25)	.02
In vivo T cell depletion						
No	1		1		1	
Yes	0.44 (0.35–0.55)	<.001	1.04 (0.96–1.19)	.23	1.26 (1.14–1.41)	<.001

Abbreviations: CI, confidence interval; CMV, cytomegalovirus; ext, extensive; GRFS, GVHD-free, relapse-free survival; HR, hazard ratio; HSCT, hematopoietic stem cell transplantation; LFS, leukemia-free survival; neutrophil engraftment, day 30 absolute neutrophil count ≥0.5 × 10^9/L; NRM, non-relapse mortality; OS, overall survival; platelet engraftment, day 30 platelet count ≥20 × 10^9/L; PTCy, post-transplant cyclophosphamide; RI, relapse incidence; TBI, total body irradiation; UD, unrelated donor.

TABLE 4 Cause of death.

	Total ($N = 2417$)	No PTCy ($N = 2226$)	PTCy ($N = 191$)
Cause of death			
GVHD	379 (16.8)	357 (17.3)	22 (11.8)
Infection	330 (14.6)	301 (14.6)	29 (15.5)
Organ toxicity	81 (3.6)	73 (3.5)	8 (4.3)
Original disease	1233 (54.7)	1123 (54.3)	110 (58.8)
Other transplant-related	163 (7.2)	151 (7.3)	12 (6.4)
Secondary malignancy	38 (1.7)	34 (1.6)	4 (2.1)
VOD	30 (1.3)	28 (1.4)	2 (1.1)
Missing	163	159	4

Note: Unless otherwise stated, results expressed as frequencies (%).
Abbreviations: GVHD, graft-versus-host disease; PTCy, post-transplant cyclophosphamide; VOD, veno occlusive disease of the liver.

LFS: HR 0.88, $p = .22$; OS: HR 0.81, $p = .09$; GRFS: HR 1.04, $p < .001$ and NRM: HR 0.64, $p = .008$ (Table S1).

3.3 | Cause of death

A total of 2417 patients died within 2 years post-transplantation, 191 (7.9%) of those receiving PTCy, and 2226 of those receiving No PTCy as GVHD prophylaxis. The original disease was the main cause of death accounting for 58.8% and 54.3% of deaths, respectively. The second cause of death was GVHD with 11.8% and 17.3%, followed by infection with 15.5% and 14.6% of deaths, in patients receiving PTCy and No PTCy, respectively. Organ toxicity accounted for 4.3% and 3.5% of the deaths, respectively. Other infrequent (≤2.1%) causes of death were second malignancies and veno-occlusive disease of the liver (Table 4).

4 | DISCUSSION

In this study, we assessed outcomes of UD-HSCT with PTCy as anti-GVHD prophylaxis, focusing on RI in a homogenous cohort of patients with AML transplanted in CR1, and observed no increase in RI with PTCy with a 2-year RI rate of about 25%. This result is very encouraging as initial reports with PTCy in the haploidentical setting indicate high RI with PTCy[14,15,33] which is a major drawback as relapse is the main cause of transplantation failure and accounts for about 50% of patient deaths beyond day 100 post-transplantation.[34–37] The initial high RI in the studies performed in early 2000s were due to the high-risk and advanced-phase disease patients included.[14] In addition, those transplants were performed with the original Baltimore platform that consisted of RIC with Cyclophospamide 14.5 mg/kg/day on days −6 and −5, flu 30 mg/m^2/day on days −6 to −2, and low dose 200 cGy TBI on day −1 and BM grafts.[14,15] In addition, as we mentioned above, the mechanism of action of PTCy includes upregulation of Treg cells and downregulation of alloreactive T cells and NK cells, raising a theoretical concern that PTCy reduces the anti-tumor efficacy of HLA-mismatched grafts.[17–19] From a clinical point of view, GVHD (especially in its chronic form) correlates with RI and besides the actual occurrence of clinical relapse, it is almost the only read-out assay for the so-called GVL effect.[5,6,8,38,39] As PTCy anti-GVHD prophylaxis resulted in a significant reduction of GVHD and even more so of cGVHD,[14,15,24] there is further theoretic support to the notion that PTCy is attributed to the high RI observed post-Haplo-HCST with its administration. However, subsequent single-center and registry-based studies that assessed transplantation outcomes as per disease risk, demonstrated that Haplo-HSCT with PTCy outcomes including RI appeared comparable to those of HLA-matched transplants with No PTCy.[24,25,39] Yet the scenario may differ in the setting of UD-HSCT, which emphasizes the importance of our finding of a 2-year RI of 24.9% with PTCy-based anti-GVHD prophylaxis in AML patients undergoing UD-HSCT. The RI we observed in the PTCy cohort is very similar to the figures of 11%–39.1% observed in the previous studies that evaluated outcomes of UD-HSCT with PTCy including previous studies from our group.[20–22,24,39–42] In this regard, Ruggeri et al. reported an incidence of 33.9% in patients with AML in a study that also included acute lymphoblastic leukemia (ALL). Sanz et al. reported an RI of 25% in a study that included various donor types, Battipaglia et al. compared PTCy to ATG as GVHD prophylaxis in 9/10 UD-HSCT with an RI of 29%, Brissot et al. performed a similar comparison in 10/10 matched UD grafts reporting an incidence of 25.2%, and Lorentino et al. observed an RI of 24% and 28% in 10/10 UD-HSCT and in 9/10 UD-HSCT, respectively.[20–22,24,42] In the HOVON-96 prospective randomized, multicenter, phase III trial that compared PTCy-based to CSA and mycophenolate mofetil GVHD prophylaxis in matched related and UD peripheral blood transplantation, the 3-year RI in the PTCy arm was 32% versus 24% in the control arm.[43] The CTN1301 multicenter phase III trial compared PTCy with BM grafts to TAC/MTX with BM grafts or CD34-selected PBSCs in patients with acute leukemia or myelodysplastic syndrome undergoing an HLA-matched HSCT with MAC. Notably, PTCy was associated with a lower incidence of disease relapse compared with controls (2 year RI of 13.9% [95% CI 8.1–21.2; HR, 0.52; 0.28–0.96; $p = .037$]).[44] Likewise, in the recently published CIBMTR randomized, multicentre

phase III trial (CTN 1703) comparing PTCy-based to tacrolimus/methotrexate GVHD prophylaxis, the cumulative RI and progression at 1 year was 20.8% in the PTCy arm versus 20.2% in the control arm.[45] The fact that PTCy seems not to abrogate the GVL effect and thus does not result in an increased risk of relapse may be attributed to data indicating that PTCy may provide a direct immune-mediated, specific anti-leukemic effect, that is probably mediated by the release of cytokines or other molecules to which leukemic cells may be more sensitive than normal cells.[21] PTCy was shown not to completely eradicate the proliferation and cytokine production of alloreactive T-cells thus maintaining the GVL effect.[46] Moreover, using modern immune profiling and machine learning techniques, unique immune signatures and T-cell subset reconstitutions were recently demonstrated with PTCy, which may allow a potent GVL effect while reducing GVHD.[47] Indeed, we observed (as did multiple previous studies) a significant reduction in the incidence of acute and cGVHD leading to lower NRM and improvement in GRFS with PTCy compared to conventional GVHD prophylaxis which is explained by the PTCy mechanism of action of down-regulating alloreactive T cells and upregulating of Treg cells.[19–24,39–47] As for the lower engraftment we observed with PTCy as compared to the conventional GVHD prophylaxis, although debatable, it is in line with some of the previous publications of results in the haploidentical setting and some recent data of higher early mixed donor chimerism with PTCy which was an independent risk factor for relapse-free survival.[48–50] Of note, our Spanish colleagues recently performed a matched-pair analysis comparing the transfusion requirements and the clinical outcomes of patients who underwent matched sibling donor peripheral blood transplantation using PTCy with a historical cohort that had received the standard CSA-based prophylaxis (100 patients in both cohorts) demonstrating that neutrophil engraftment was significantly delayed in the PTCy group compared with the CSA group (16 vs. 13 days; $p = .003$) and that PTCy was associated with significantly increased red blood cells and platelet transfusion requirements during the first 30 days after transplantation.[51]

The additional prognostic factors we observed for predicting relapse and transplantation outcome including adverse cytogenetics, female donor to male patient combination, increasing age, mismatched versus matched UDs, and patient CMV seropositivity were previously reported as prognostic factors for the outcome of transplantation in AML including with PTCy GVHD prophylaxis.[21,22,36] This current retrospective study is transplantation registry-based and thus has several limitations including potential selection bias, and the possibility of unavailable data (such as molecular, and measurable residual disease) that have not been considered. We also lacked information on pre-transplant frontline treatments and the hematopoietic cell transplantation-specific comorbidity index.

In conclusion, in this registry-based, retrospective analysis of UD-HSCT with PTCy compared *to* No PTCy GVHD prophylaxis in patients with AML in CR1, we observed no significant difference in RI, while aGVHD, cGVHD, and NRM were significantly lower, leading to significantly higher GRFS with PTCy compared to No PTCy. Clinically, these results are of importance, and although not direct proof, they may indicate that PTCy does not jeopardize the GVL effect in the UD setting. A well-designed, randomized two-arm study with RI as the primary endpoint in a homogenous cohort of AML patients undergoing UD transplantation with PTCy is warranted.

AUTHOR CONTRIBUTIONS

AN wrote the manuscript, designed the study, and interpreted the data. MN, J-EG, and ML performed the statistical analyses, interpreted the data, and edited the manuscript. IWB, NK, TG-D, TS, DB, US, AR, GC, RPdL, JV, HS, ME, SM, EF, AK, and FC reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no relevant conflict of interest and no competing financial interests.

DATA AVAILABILITY STATEMENT

AN, MN, FC, and MM had full access to all study data (available upon data-specific request).

PATIENT CONSENT STATEMENT

The authors have nothing to report.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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