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ORIGINAL PAPER

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Outcomes of children with haematological malignancies given second haploidentical haematopoietic stem cell transplantation with either TCRαβ/CD19 depletion or post-transplant cyclophosphamide

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Summary

Human leukocyte antigen (HLA)-haploidentical haematopoietic cell transplantation (haplo-HCT) is a suitable salvage strategy in children with haematological malignancies experiencing either relapse or graft failure (GF) after the first HCT. Data comparing outcomes of transplant strategies using either TCRαβ/CD19 depletion (TCRαβ) or post-transplant cyclophosphamide (PTCy) are currently lacking. This retrospective, multicentre study included children with haematological malignancies who received a second haplo-HCT, in which either TCRαβ depletion or PTCy was used as the graft-versus-host disease (GvHD) prophylaxis strategy. Primary outcomes included overall survival (OS), event-free survival (EFS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM). Overall, 123 patients were analysed, 56 receiving PTCy and 67 receiving TCRαβ. Median age at transplant was 9.1 years (range, 1.0–24.7 years). Relapse and GF were the transplant indications in 96 and 27 patients respectively. The 24-month OS [56.8% (95% CI: 42.5%–71.1%) vs. 43.2%

(95% CI: 31.4%–55.1%)] and EFS [44.1% (95% CI: 30.3%–57.8%) vs. 35.8% (95% CI: 24.3%–47.3%)] did not differ between PTCy or TCRαβ cohorts. The CIR [34.0% (95% CI: 23.1%–50.1%) vs. 37.3% (95% CI: 27.3%–50.8%), $p = 0.28$] and NRM [18.9% (95% CI: 10.7%–33.4%) vs. 25.3% (95% CI: 16.8%–38.2%), $p = 0.48$] were comparable. Cumulative incidence of 100-day of any-grade acute GvHD was higher in the PTCy cohort [55.3% (95% CI: 43.7%–70.4%) vs. 32.8% (95% CI: 23.3%–46.2%), $p = 0.02$], with non-statistically significant differences for grade II–IV and grade III–IV. The 24-month cumulative incidence of chronic GvHD was higher in the PTCy cohort [38.8% (95% CI: 27.6%–54.6%) vs. 11.9% (95% CI: 6.2%–22.8%), $p < 0.01$], including moderate–severe forms [15.3% (95% CI: 8.1%–29.1%) vs. 1.4% (95% CI: 0.2%–10.4%), $p < 0.01$]. Infectious complications were comparable except for a higher adenovirus reactivation rate in the TCRαβ group (14.3% vs. 29.9%, $p = 0.04$). PTCy and TCRαβ offer comparable clinical outcomes in the setting of second haplo-HCT, although PTCy is associated with a higher incidence of GvHD and lower adenovirus reactivation.

KEYWORDS
children, GvHD, haploidentical, PTCy, second HCT, TCRαβ/CD19

INTRODUCTION

Allogeneic haematopoietic cell transplantation (HCT) is an effective treatment for many high-risk or relapsed paediatric haematological malignancies (HM).[1–4] The outcome of HCT in children has progressively improved over the years, due to advancements in transplant techniques.[5,6] However, some patients experience a treatment failure, mainly due to post-transplant disease relapse[7,8] or, less frequently, to either primary or secondary graft failure (GF).[9] Both disease recurrence and GF occur in a variable percentage of patients, mainly depending on the disease phase/status and the transplant characteristics, and have a significantly negative impact on patient outcomes.[10] A second allogeneic HCT is a potential therapeutic option,[11,12] though it presents several challenges, including the availability of a suitable donor, a higher risk of transplant-related complications and an increased likelihood of immune-mediated complications.[13] In this setting, HLA-haploidentical haematopoietic cell transplantation (haplo-HCT) is a suitable strategy for a second procedure, considering the frequent availability of one or more rapidly accessible familial donors. To overcome the HLA barrier and manage the increased risk of graft-versus-host disease (GvHD) associated with haploidentical donors, two different approaches have been developed for paediatric patients in recent years, namely, selective TCRαβ/CD19-depletion (TCRαβ) and T-cell modulation/depletion (TCMD) with post-transplant cyclophosphamide (PTCy). The former consists in an ex vivo selective depletion of αβ T and B cells in the graft, enabling the transfer of donor stem cells, committed haematopoietic progenitors and relevant numbers of donor mature NK and γδ T cells, able to potentially confer protection against pathogens and leukaemia cell regrowth.[14,15] In contrast, the PTCy strategy, initially developed by the Johns Hopkins group,[16,17] uses cyclophosphamide to selectively target proliferating lymphocytes, depleting alloreactive cells while sparing pathogen-specific, memory experienced T cells and regulatory T cells, thereby promoting engraftment, conferring protection against infectious complications and reducing GvHD.[17] Both strategies have been shown to be feasible and effective in children.[15,18–21] However, no comparative data are currently available to guide the choice of transplant strategy in the context of a second haplo-HCT. Our study primarily aimed to assess the feasibility and outcomes of paediatric patients with haematological malignancies given second haplo-HCT with either TCRαβ or PTCy for both GF and disease recurrence, to inform clinical decision-making in this challenging setting.

METHODS

Study design and participants

This is a retrospective, multicentre, international analysis on paediatric patients with haematological malignancies who underwent a second haplo-HCT due to either disease relapse or GF. The study was approved by the local ethic committee by each institution. The inclusion criteria for the study were as follows: patients aged between 0 and 25 years; transplant procedures performed between 1 January 2011 and 31 December 2022; indication for a second transplantation due to primary or secondary GF or disease relapse; availability of a haploidentical family donor; GvHD prophylaxis strategies based on either TCRαβ or PTCy, according to the centre's policy; a previous transplantation from any donor type.

Transplantation procedures

Transplant procedure was performed according to the centres' policy. PTCy was administered on day +3+4 or +3+5 according to the centres' common practice, with the dose

of 50 mg/kg/dose. TCRαβ/CD19-depletion was performed as previously reported.[15] In brief, it was conducted using a closed system with clinical grade reagents and Miltenyi Biotec instrumentation (Bergish-Gladbach, Germany). Briefly, apheresis products were tagged with biotin-conjugated anti-TCRαβ antibodies, followed by labelling with paramagnetic beads conjugated to anti-biotin and anti-CD19 antibodies for B-cell depletion. The CliniMACS device processed the cells through a magnetic column, and aliquots of the final graft were analysed for CD34+ HSCs, TCRγδ, NK cell content, as well as for the assessment of residual TCRαβ T and B cells. The use of granulocyte-colony stimulating factor (G-CSF) followed each centre's internal policy. Regardless of the GvHD prophylaxis regimen, some centres administered G-CSF starting the day after the second dose of cyclophosphamide until neutrophil engraftment, while others did not routinely use G-CSF and reserved its administration for delayed neutrophil recovery or in the presence of complications such as infections.

End-points and definitions

Primary outcomes included overall survival (OS), event-free survival (EFS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM) in patients receiving either TCRαβ or PTCy. Secondary outcomes included GvHD-free, relapse-free survival (GRFS), cumulative incidence of GF, acute GvHD (aGvHD), chronic GvHD (cGvHD), any type of infections, transplant-related toxicities and immune reconstitution at 30- and 100-day post-haplo-HCT. GF was defined according to the updated European Bone Marrow Transplant (EBMT) criteria.[22] aGvHD was graded according to the Glucksberg criteria.[22,23] cGvHD was defined and classified according to the National Institutes of Health (NIH) criteria.[24] Neutrophil engraftment was defined as the first of three consecutive days in which the neutrophil count reached or exceeded 0.5×10^9/L. Platelet engraftment was defined as the first day in which a platelet count of $\geq 20 \times 10^9$/L was achieved without a platelet transfusion for at least 7 days.

Statistical analysis

Collected data were reported using descriptive statistics. Continuous variables were reported by their median value, range or standard deviation (SD). The variables were compared between patients receiving TCRαβ or PTCy using the Wilcoxon rank sum test for continuous variables and the chi-squared test for categorical variables. OS, EFS and GRFS were estimated using the Kaplan–Meier method, while CIR and NRM were estimated using the cumulative incidence function to account for competing risks. Log-rank and Grey's test were used to compare the data between the two groups. *p*-value was considered statistically significant if <0.05. Statistical analyses were performed using NCSS (NCSS 12 Statistical Software (2018). NCSS, LLC., Kaysville, UT, USA).

RESULTS

Patients and transplant characteristics

A total of 123 patients from nine transplant centres were included. Median age at second transplant was 9.2 years (range, 1.0–24.7 years). Patients' characteristics are reported in Table 1. Patients were predominately males (63.4%) with acute lymphoblastic leukaemia and acute myelogenous leukaemia as more frequent diagnoses, in 44.7% and 39.8% of cases respectively. Patients received a median of 3 (range, 2–5) lines of therapy including the first HCT before the haplo-HCT. Relapse and GF were the indications for the second transplant in 96 (78.0%) and 27 (22.0%) cases respectively. Haplo-HCT was performed at a median of 12.6 months (range, 0.9–94.8) after the first and at a median of 3.6 months (range, 0.3–28.5) after the relapse or GF. Patients with GF and relapse received the second transplant after a median of 0.7 and 4.0 months respectively ($p < 0.001$). As GvHD prophylaxis, 56 patients received PTCy, with bone marrow as the stem cell source in 80.4%, while 67 patients received TCRαβ, all with peripheral blood as the stem cell source. Conditioning regimens varied among the patients and were TBI-based, treosulfan-based and busulfan-based in 26.0%, 31.7% and 24.4% of patients respectively. Median number of infused CD34+ cells/kg was 9.39×10^6/kg (range, 1.40–33.50), with a higher median number in TCRαβ than in PTCy (15.50 vs. 4.98, $p < 0.001$). For patients receiving PTCy, the median number of infused CD3+ was 0.54×10^8/kg (range, 0.00–4.53). Patients receiving PTCy as GvHD prophylaxis also received a calcineurin inhibitor and mycophenolate mofetil, starting the day after the second dose of PTCy. In three cases, anti-thymocyte globulin was added to the PTCy protocol. Patients in the TCRαβ groups have not received any post-transplant pharmacologic GVHD prophylaxis. When comparing the baseline characteristics of the PTCy and TCRαβ groups, patients in the former group received a higher median number of lines of therapy before haplo-HCT (3 vs. 2, $p = 0.004$). The median follow-up for surviving patients was 45.3 months (range, 1.9–153.7) with a longer median follow-up in the PTCy group (64.2 vs. 36.8 months, $p = 0.002$).

Engraftment and immune reconstitution

In the PTCy and TCRαβ groups, 53 (94.6%) and 62 (92.5%) of patients, respectively, achieved neutrophil engraftment ($p = 0.63$), with a shorter time to engraftment in the TCRαβ group (16.5 ± 3.8 vs. 12.0 ± 3.5 days, $p < 0.001$) (Table 2). A higher proportion of patients in the PTCy group received G-CSF; the growth factor was also administered for more days in this group than in patients given a TCRαβ/B-cell depleted allograft (Table 2). A shorter platelet engraftment time was also observed in the TCRαβ group (11.0 ± 9.0 vs. 24.0 ± 23.5 days, respectively, $p < 0.001$). In patients receiving a second haplo-HCT for GF as transplant indication, a

TABLE 1 Characteristics of the patients' cohorts.

	Overall (*N* = 123)	PTCy (*N* = 56)	TCRαβ (*N* = 67)	*p*-value
Age at 2nd HCT—year—median (SD)	9.17 ± 5.25	9.34 ± 5.17	8.91 ± 5.34	0.683
Gender—No. (%)				0.847
Male	78 (63.4)	35 (62.5)	43 (64.2)	
Female	45 (36.6)	21 (37.5)	24 (35.8)	
Diagnosis—No. (%)				0.079
ALL	55 (44.7)	30 (53.6)	25 (37.3)	
MDS	6 (4.9)	4 (7.1)	2 (3)	
MPAL	4 (3.3)	1 (1.8)	3 (4.5)	
AML	49 (39.8)	15 (26.8)	34 (50.7)	
JMML	9 (7.3)	6 (10.7)	3 (4.5)	
Median number of previous lines of therapy—No. (range)	3 (2–5)	3 (2–5)	2 (2–4)	**0.004**
2nd HCT indication—No. (%)				0.898
Relapse	96 (78)	44 (78.6)	52 (77.6)	
Graft failure	27 (22)	12 (21.4)	15 (22.4)	
Time from 1st to 2nd HCT—months—median (SD)	12.56 ± 25.20	12.93 ± 16.41	12.56 ± 14.20	0.505
Time from event to 2nd HCT—months—median (SD)	3.60 ± 4.30	3.42 ± 3.49	3.83 ± 4.87	0.769
Stem cell source—No. (%)				**<0.001**
BM	45 (36.6)	45 (80.4)	0 (0)	
PBSC	78 (63.4)	11 (19.6)	67 (100)	
Conditioning regimen—No. (%)				0.304
TBI-based	32 (26)	11 (19.6)	21 (31.3)	
Treosulfan-based	39 (31.7)	21 (37.5)	18 (26.9)	
Busulfan-based	30 (24.4)	18 (32.1)	12 (17.9)	
Other	22 (17.9)	6 (10.8)	16 (23.9)	
CD34+ $n/10^6$/kg—median (SD)	9.49 ± 7.57	4.98 ± 4.50	15.50 ± 6.25	**<0.001**
CD3+—($n/10^8$/kg)—median (SD)		0.54 ± 1.21		
GvHD prophylaxis—No. (%)				**<0.001**
TCMD	64 (52)	0 (0)	64 (95.5)	
TCMD, Sirolimus, MMF	3 (2.4)	0 (0)	3 (4.5)	
PTCy, CSA, MMF, ATG	3 (2.4)	3 (5.4)	0 (0)	
PTCy, CSA, MMF	42 (34.2)	42 (75)	0 (0)	
PTCy, FK506, MMF	11 (9.0)	11 (19.6)	0 (0)	
G-CSF administration—No. (%)	53 (48.6)	30 (53.5)	23 (34.3)	**<0.001**
G-CSF administration—days—median (range)	10 (0–27)	12 (0–25)	6 (0–27)	**<0.001**

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; ATG, anti-thymocyte globulin; BM, bone marrow; CMML, chronic myelomonocytic leukaemia; CSA, ciclosporin A; G-CSF, granulocyte-colony stimulating factor; GvHD, graft-versus-host disease; JMML, Juvenile myelomonocytic leukaemia; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MPAL, mixed phenotype acute leukaemia; PBSC, peripheral blood stem cells; PTCy, post-transplant cyclophosphamide; TBI, total body irradiation; TCMD, T-cell depletion; TCRαβ, TCRαβ/CD19-depletion.

Note: Bold values indicate statistically significant ($p < 0.05$).

successful engraftment was achieved in 25/27 (92.6%) patients. No differences in total lymphocyte counts were found at day +30 in the PTCy and TCRαβ groups (425/μL vs. 395/μL, $p = 0.45$) and day +100 (869/μL vs. 900/μL, $p = 0.34$) and in CD4+ T-cell counts at day +30 (58/μL vs. 78/μL, $p = 0.29$) and day +100 (116/μL vs. 170/μL, $p = 0.21$).

Outcomes

The 24-month OS and EFS did not differ between the PTCy or TCRαβ cohorts [56.8% (95% CI: 42.5%–71.1%) vs. 43.2% (95% CI: 31.4%–55.1%), $p = 0.11$; EFS 44.1% (95% CI: 30.3%–57.8%) vs. 35.8% (95% CI: 24.3%–47.3%), $p = 0.22$] (Figure 1). The

TABLE 2 Engraftment and reconstitution data.

	Overall ($N=123$)	PTCy ($N=56$)	TCRαβ ($N=67$)	p-value
Primary graft failure	8 (6.5)	3 (5.4)	5 (7.5)	0.637
Neutrophil engraftment—day—median (SD)	15.0 ± 4.1	16.5 ± 3.8	12.0 ± 3.5	*<0.001*
Platelet (20k) engraftment—day—median (SD)	14.0 ± 18.3	24.0 ± 23.5	11.0 ± 9.0	*<0.001*
Platelet (50k) engraftment—day—median (SD)	14.0 ± 72.0	91.5 ± 70.5	13.5 ± 71.0	0.117
TLC at day +30—median (range)	425 (0–5780)	395 (0–3480)	460 (10–5780)	0.452
TLC at day +100—median (range)	869 (30–5140)	900 (30–3950)	820 (60–5140)	0.341
CD4+ at day +30—median (range)	58 (0–1530)	78 (0–1530)	33 (4–836)	0.292
CD4+ at day +100—median (range)	116 (7–1200)	170 (10–1200)	82 (7–695)	0.211

Abbreviations: PTCy, post-transplant cyclophosphamide; TCRαβ, TCRαβ/CD19-depletion; TLC, total lymphocyte count.

Note: Bold values indicate statistically significant ($p < 0.05$).

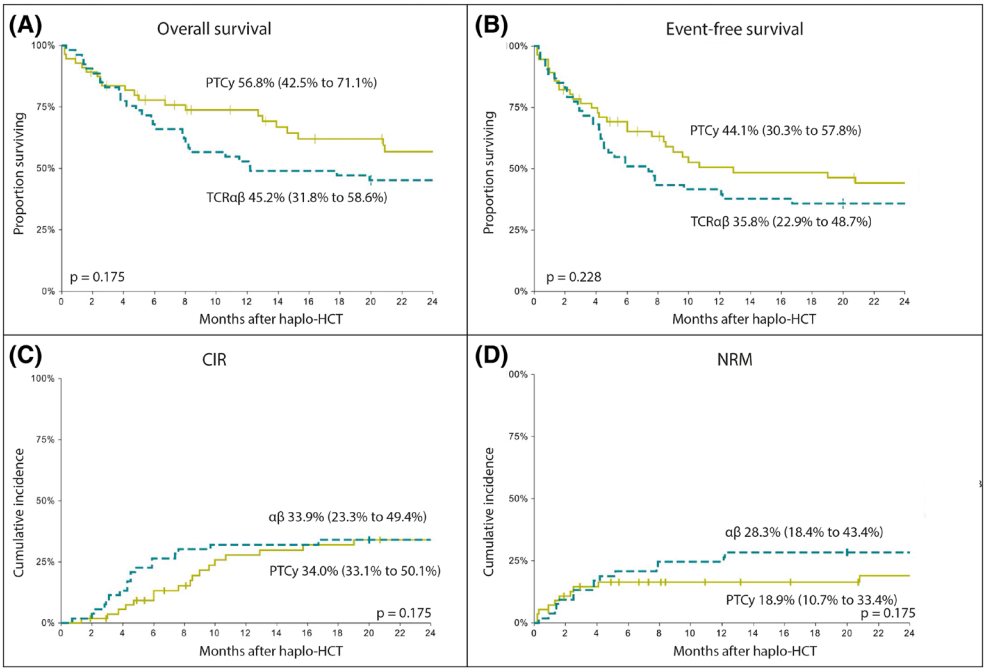

FIGURE 1 Overall survival, event-free survival, cumulative incidence of non-relapse mortality and relapse according to T-cell depletion method. (A) Kaplan–Meier curves of OS stratified by TCMD; (B) Kaplan–Meier curves of EFS stratified by TCMD; (C) cumulative incidence of relapse stratified by TCMD; (D) cumulative incidence of relapse stratified by TCMD. EFS, event-free survival; OS, overall survival; TCMD, T-cell modulation/depletion. [Colour figure can be viewed at wileyonlinelibrary.com]

CIR [34.0% (95% CI: 23.1%–50.1%) vs. 37.3% (95% CI: 27.3%–50.8%), $p=0.28$] and NRM [18.9% (95% CI: 10.7%–33.4%) vs. 25.3% (95% CI: 16.8%–38.2%), $p=0.48$] were also comparable between PTCy and TCRαβ. The cumulative incidence of 100-day any-grade acute GvHD was higher in the PTCy cohort [55.3% (95% CI: 43.7%–70.4%) vs. 32.8% (95% CI: 23.3%–46.2%), $p=0.02$], with non-significant differences observed for grade II–IV [42.8% (95% CI: 31.6%–57.9%) vs. 25.3% (95% CI: 16.8%–38.2%), $p=0.08$] and grade III–IV [12.5% (95% CI: 6.2%–25.0%) vs. 8.9% (95% CI: 4.1%–19.2%), $p=0.75$] (Figure 2). The cumulative incidence of gut acute GvHD was higher in the PTCy than in the TCRαβ cohort (Figure 2). The 24-month cumulative incidence of chronic GvHD was higher in the PTCy cohort [38.8% (95% CI: 27.6%–54.6%) vs. 11.9% (95% CI: 6.2%–22.8%), $p<0.001$], including moderate–severe forms [15.3% (95% CI: 8.1%–29.1%) vs. 1.4% (95% CI: 0.2%–10.4%), $p=0.004$]. The 24-month GRFS was lower in the PTCy group than in the TCRαβ [5.5% (95% CI: 0.0%–11.5%) vs. 19.4% (95% CI: 9.9%–28.8%), $p=0.008$]. When stratifying patients according to the indication to the second transplant, in patients receiving a second haplo-HCT for relapse (median follow-up of 41.4 months, range: 1.9–141.9), outcomes were similar between the PTCy and TCRαβ groups [24-month OS: 48.4% (95% CI: 31.6%–65.3%) vs. 36.5% (95% CI: 23.4%–49.6%), $p=0.10$; 24-month EFS: 34.0% (95% CI: 18.7%–49.2%) vs. 30.7% (95% CI: 18.2%–43.3%), $p=0.35$; 24-month CIR: 45.0% (95% CI: 31.6%–64.0%) vs. 42.3% (95% CI: 30.8%–58.1%), $p=0.57$; 24-month NRM: 19.5% (95% CI: 10.3%–36.8%) vs. 26.9% (95% CI: 17.2%–42.1%), $p=0.48$]. Similarly, no significant differences were observed in the GF subgroup (median follow-up

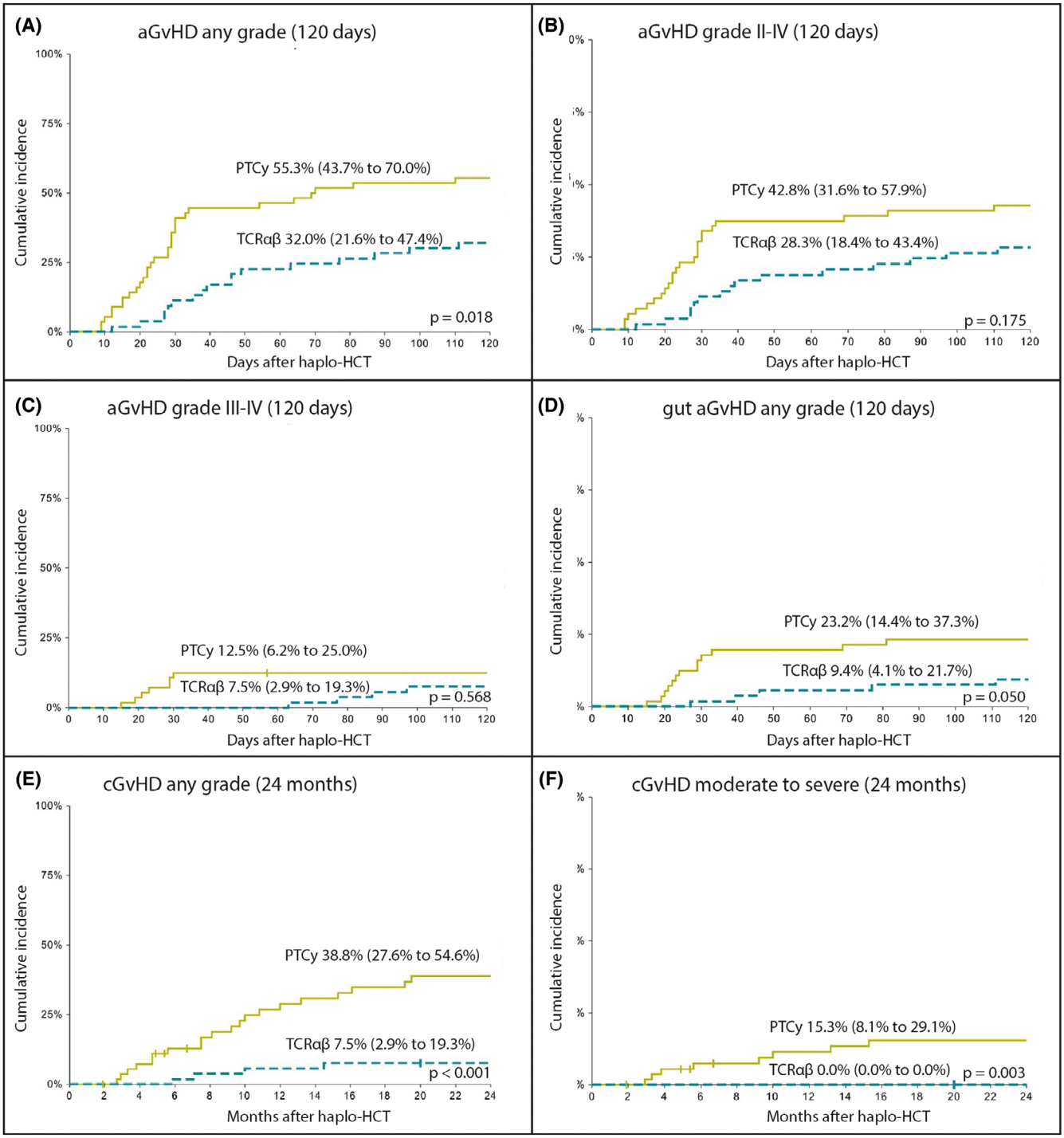

FIGURE 2 GvHD incidence according to T-cell depletion method. Cumulative incidence according to TCMD for (A) any grade aGvHD; (B) grade II–IV aGvHD; (C) grade III–IV aGvHD; (D) gut aGvHD; (E) any grade cGvHD; (F) moderate to severe cGvHD. aGvHD, acute graft-versus-host disease; GvHD, graft-versus-host disease; TCMD, T-cell modulation/depletion. [Colour figure can be viewed at wileyonlinelibrary.com]

of 60.8 months, range: 3.3–153.7) [24-month OS: 82.5% (95% CI: 60.4%–100.0%) vs. 66.6% (95% CI: 42.8%–90.5%), $p = 0.81$; 24-month EFS: 75.0% (95% CI: 50.5%–99.5%) vs. 53.3% (95% CI: 28.0%–78.5%), $p = 0.59$; CIR: 0.0% (95% CI: 0.0%–0.0%) vs. 20.0% (95% CI: 7.2%–55.0%), $p = 0.11$; 24-month NRM: 17.5% (95% CI: 4.9%–61.8%) vs. 20.0% (95% CI: 7.2%–55.0%), $p = 0.91$].

Transplant complications

Transplant complications of patients receiving PTCy or TCRαβ as GvHD prophylaxis are reported in detail in Table 3. Infectious complications occurred in 62.5% and 77.6% of patients in the PTCy and TCRαβ groups, respectively, with no significant differences ($p = 0.07$). Bacterial infections had a

TABLE 3 Transplant complications.

	Overall ($N=123$)	PTCy ($N=56$)	TCRαβ ($N=67$)	p-value
Patients with infections—No. (%)	87 (70.7)	35 (62.5)	52 (77.6)	0.067
Total infection events—No.	240	97	143	0.342
Bacterial infection—No. (%)	40 (32.5)	18 (32.1)	22 (32.8)	0.935
Bacterial infection period—No. (%)				0.471
First 100 days	23 (57.5)	10 (55.5)	13 (59.1)	
100–180 days	8 (20)	5 (27.8)	3 (13.6)	
181–365 days	9 (22.5)	3 (16.7)	6 (27.3)	
Bacterial infection localization—No. (%)				0.641
BSI	32 (80)	14 (77.6)	18 (81.9)	
Pneumonia	3 (7.5)	1 (5.6)	2 (9.1)	
GI	2 (5)	1 (5.6)	1 (4.5)	
Cutaneous	1 (2.5)	1 (5.6)	0 (0)	
CNS	1 (2.5)	0 (0)	1 (4.5)	
UTI	1 (2.5)	1 (5.6)	0 (0)	
CMV reactivation by day 180—No. (%)	48 (39)	22 (39.3)	26 (38.8)	0.957
Fungal infections—No.	15 (12.2)	6 (10.7)	9 (13.4)	0.646
Candida spp.	4 (3.3)	2 (3.6)	2 (3)	
Aspergillus spp.	11 (8.9)	4 (7.1)	7 (10.4)	
Viral infections—No.	72 (58.5)	28 (50)	44 (65.7)	0.079
EBV reactivation	11 (8.9)	9 (16.1)	2 (3)	
Adenovirus reactivation	28 (22.8)	8 (14.3)	20 (29.9)	
Other toxicities				
MOF	17 (13.8)	5 (8.9)	12 (17.9)	0.151
VOD/SOS	8 (6.5)	3 (5.4)	5 (7.5)	0.637
Haemorrhagic cystitis related to BK	17 (13.8)	6 (10.7)	11 (16.4)	**0.011**

Abbreviations: BSI, bloodstream infection; CMV, cytomegalovirus; CNS, central nervous system; EBV, Epstein–Barr virus; GI, gastrointestinal; MOF, multi-organ failure; PTCy, post-transplant cyclophosphamide; TCRαβ, TCRαβ/CD19-depletion; VOD/SOS, sinusoidal obstruction syndrome.

Note: Bold values indicate statistically significant ($p < 0.05$).

similar incidence in the two cohorts, mainly occurring in the first 100 days after transplant, with no significant differences in the localization. Cytomegalovirus reactivations by day 180 were comparable in the two groups (39.3% vs. 38.8%, $p = 0.957$). Of note, none of the included patients was on letermovir prophylaxis. A significantly higher incidence of adenovirus reactivation was observed in the TCRαβ group (14.3% vs. 29.9%, $p = 0.040$). Regarding transplant toxicities, no differences were found in the rate of veno-occlusive disease (VOD/SOS), but a significantly higher incidence of haemorrhagic cystitis related to BK virus was found in the TCRαβ group (10.7% vs. 16.4%, $p = 0.011$).

DISCUSSION

We herein report the first comparison of two widely used methods for GvHD prophylaxis in children with haematological malignancies undergoing a second haplo-HCT for either GF or disease recurrence. Only limited data compare the two most widely used approaches, namely PTCy and TCRαβ/B-cell depletion for the first transplant, and no data are available in the context of a second transplant.[25] Our results underline the feasibility of both procedures in the setting of a second transplant with a high rate of engraftment even in patients with previous GF. Time to engraftment was consistent with that reported in the literature for TCRαβ[15] and PTCy[20] and resulted shorter for the TCRαβ method. However, despite the earlier engraftment in TCRαβ, there was no observed impact on the incidence of severe infections within the first 100 days. Moreover, no other differences were found in the post-transplant total and CD4+ lymphocyte count after haplo-HCT, consistent with literature data.[15,18] Another notable finding is that patients experiencing GF after the first HCT underwent a second haplo-HCT after a median of 0.66 months after the GF diagnosis, underscoring the possibility to rapidly proceed with a second procedure. NRM was acceptable in both groups considering that patients were heavily pretreated and received a previous transplant and was comparable with previous reports reporting transplant procedures from several type of donor.[26] Fierro-Pineda et al.[20] previously reported a 12-month rate

of transplant-related mortality of 0%; however, patients included in that study underwent the first haplo-HCT and cumulative incidence of relapse was higher than the one reported in our cohort. OS and EFS in our two groups were comparable and aligned with data on second transplant procedures for paediatric acute leukaemias,[26] as well as the CIR which was similar in patients receiving the haplo-HCT for both GF or relapse after the first. Any-grade aGvHD was significantly more frequent in the PTCy cohort, although grades II–IV and III–IV showed a trend towards higher incidence without reaching statistical significance. Concerning cGvHD, the PTCy group exhibited a significantly higher rate of any grade and moderate to severe forms. These data underline a significant incidence of cGvHD and align with previous reports.[20] Indeed, patients undergoing haplo-HCT with PTCy as the TCMD method may require enhanced prophylaxis for cGvHD, such as the addition of a low dose of anti-thymocyte globulin (ATG) to PTCy[27,28] or other therapies, including mesenchymal stem cells,[29] ruxolitinib[30] or abatacept,[31] tailored on the risk of post-transplant relapse. While in our cohort the incidence of cGvHD was significantly higher in the PTCy group, considering the comparable CIR, it can be speculated that the immunosuppression required to treat GvHD may not impact disease control, but further data are needed to confirm this hypothesis.

Regarding infectious complications, the two groups generally presented a comparable incidence of such complications. A higher rate of adenovirus reactivations was found in the TCRαβ group, which aligns with the previously published literature.[15] Limitations of the analysis of infectious complications include the lack of assessment of their clinical relevance, such as the treatment needs and costs, and the absence of data on antibacterial prophylaxis. A slightly higher incidence of haemorrhagic cystitis related to BK was found in the TCRαβ as well, despite the fact that this complication is usually reported to be associated with the use of PTCy.[32] Indeed, the low number of these events limits the power of the study to specifically address this issue that should be assessed in future studies. This study had some limitations. First, it included patients receiving haplo-HCT after experiencing either relapse or graft failure, which, while providing shared feasibility and outcomes data, may involve different clinical approaches. Moreover, the two cohorts presented some baseline differences, such as differences in the median number of lines of therapy before haplo-HCT, in the median follow-up for surviving patients and in G-CSF administration.

CONCLUSION

Both PTCy and TCRαβ are valuable strategies for GvHD prophylaxis in second haplo-HCT, demonstrating comparable outcomes, including CIR. However, PTCy is associated with a higher incidence of GvHD and a lower incidence of adenovirus reactivations.

AUTHOR CONTRIBUTIONS

R.M., D.L., F.G., F.L. and D.P.: Conceptualization. G.A.M.O., F.V., F.S., F.P., M.A., F.D.B., P.M., A.P., S.C., M.U., M.F., M.Z., F.F., A.B. and J.H.D.: Data curation. D.L. and F.G.: Formal analysis. D.L., F.G. and F.B.: Roles/writing—original draft. R.M., F.L. and D.P.: Writing—review and editing, and supervision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data supporting this study are openly available in Zenodo with code https://doi.org/10.5281/zenodo.15001057, under the applicable licence.

ETHICS APPROVAL STATEMENT

The study was approved by the local Ethic committee by each institution.

PATIENT CONSENT STATEMENT

Written parental and patient consent had been obtained by the contributing centres.

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REFERENCES

1. Copelan EA, Chojecki A, Lazarus HM, Avalos BR. Allogeneic hematopoietic cell transplantation; the current renaissance. Blood Rev. 2019;34:34–44.
2. Locatelli F, Masetti R, Rondelli R, Zecca M, Fagioli F, Rovelli A, et al. Outcome of children with high-risk acute myeloid leukemia given autologous or allogeneic hematopoietic cell transplantation in the aieop AML-2002/01 study. Bone Marrow Transplant. 2015;50:181–8.
3. Merli P, Pagliara D, Mina T, Bertaina V, Li Pira G, Lazzaro S, et al. αβT- and B-cell-depleted HLA-haploidentical hematopoietic stem cell transplantation in children with myelodysplastic syndromes. Haematologica. 2022;107:2966–71.
4. Peters C, Dalle J-H, Locatelli F, Poetschger U, Sedlacek P, Buechner J, et al. Total body irradiation or chemotherapy conditioning in childhood ALL: a multinational, randomized, noninferiority phase III study. J Clin Oncol. 2021;39:295–307.
5. Bader P, Pötschger U, Dalle J-H, Moser LM, Balduzzi A, Ansari M, et al. Low rate of nonrelapse mortality in under-4-year-olds with ALL given chemotherapeutic conditioning for HSCT: a phase 3 FORUM study. Blood Adv. 2024;8:416–28.

6. Gooley TA, Chien JW, Pergam SA, Hingorani S, Sorror ML, Boeckh M, et al. Reduced mortality after allogeneic hematopoietic-cell transplantation. N Engl J Med. 2010;363:2091–101.
7. Parikh SH, Satwani P, Ahn KW, Sahr NA, Fretham C, Abraham AA, et al. Survival trends in infants undergoing allogeneic hematopoietic cell transplant. JAMA Pediatr. 2019;173:e190081.
8. Yaniv I, Krauss AC, Beohou E, Dalissier A, Corbacioglu S, Zecca M, et al. Second hematopoietic stem cell transplantation for post-transplantation relapsed acute leukemia in children: a retrospective EBMT-PDWP study. Biol Blood Marrow Transplant. 2018;24:1629–42.
9. Locatelli F, Lucarelli B, Merli P. Current and future approaches to treat graft failure after allogeneic hematopoietic stem cell transplantation. Expert Opin Pharmacother. 2014;15:23–36.
10. Hazar V, Tezcan Karasu G, Öztürk G, Küpesiz A, Aksoylar S, Özbek N, et al. Prognostic factors for survival in children who relapsed after allogeneic hematopoietic stem cell transplantation for acute leukemia. Pediatr Transplant. 2021;25:e13942.
11. Vinci L, Flotho C, Noellke P, Lebrecht D, Masetti R, de Haas V, et al. Second allogeneic stem cell transplantation can rescue a significant proportion of patients with JMML relapsing after first allograft. Bone Marrow Transplant. 2023;58:607–9.
12. Yerushalmi Y, Shem-Tov N, Danylesko I, Canaani J, Avigdor A, Yerushalmi R, et al. Second hematopoietic stem cell transplantation as salvage therapy for relapsed acute myeloid leukemia/myelodysplastic syndromes after a first transplantation. Haematologica. 2022;108:1782–92.
13. Penack O, Abouqateb M, Peczynski C, Boreland W, Kröger N, Zeiser R, et al. How risky is a second allogeneic stem cell transplantation? Leukemia. 2024;38:1799–807.
14. Li Pira G, Malaspina D, Girolami E, Biagini S, Cicchetti E, Conflitti G, et al. Selective depletion of αβ T cells and B cells for human leukocyte antigen-haploidentical hematopoietic stem cell transplantation. A three-year follow-up of procedure efficiency. Biol Blood Marrow Transplant. 2016;22:2056–64.
15. Merli P, Algeri M, Galaverna F, Bertaina V, Lucarelli B, Boccieri E, et al. TCRαβ/CD19 cell–depleted HLA-haploidentical transplantation to treat pediatric acute leukemia: updated final analysis. Blood. 2024;143:279–89.
16. Luznik L, O'Donnell PV, Fuchs EJ. Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical bone marrow transplantation. Semin Oncol. 2012;39:683–93.
17. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008;14:641–50.
18. Berger M, Lanino E, Cesaro S, Zecca M, Vassallo E, Faraci M, et al. Feasibility and outcome of haploidentical hematopoietic stem cell transplantation with post-transplant high-dose cyclophosphamide for children and adolescents with hematologic malignancies: an AIEOP-GITMO retrospective multicenter study. Biol Blood Marrow Transplant. 2016;22:902–9.
19. Dufort G, Castillo L, Pisano S, Castiglioni M, Carolina P, Andrea I, et al. Haploidentical hematopoietic stem cell transplantation in children with high-risk hematologic malignancies: outcomes with two different strategies for GvHD prevention. Ex vivo T-cell depletion and post-transplant cyclophosphamide: 10 years of experience at a single center. Bone Marrow Transplant. 2016;51:1354–60.
20. Fierro-Pineda JC, Tsai H-L, Blackford A, Cluster A, Caywood E, Dalal J, et al. Prospective PTCTC trial of myeloablative haplo-BMT with posttransplant cyclophosphamide for pediatric acute leukemias. Blood Adv. 2023;7:5639–48.
21. Locatelli F, Merli P, Pagliara D, Li Pira G, Falco M, Pende D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion. Blood. 2017;130:677–85.
22. Sureda A, Corbacioglu S, Greco R, Kröger N, Carreras E, editors. The EBMT handbook: hematopoietic cell transplantation and cellular therapies. 8th ed. Cham (CH): Springer; 2024. Available from: http://www.ncbi.nlm.nih.gov/books/NBK608238/.
23. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18:295–304.
24. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. Biol Blood Marrow Transplant. 2015;21:389–401.e1.
25. Lum SH, Albert MH, Gilbert P, Sirait T, Algeri M, Muratori R, et al. Outcomes of HLA-mismatched HSCT with TCRαβ/CD19 depletion or post-HSCT cyclophosphamide for inborn errors of immunity. Blood. 2024;144:565–80.
26. Lund TC, Ahn KW, Tecca HR, Hilgers MV, Abdel-Azim H, Abraham A, et al. Outcomes after second hematopoietic cell transplantation in children and young adults with relapsed acute leukemia. Biol Blood Marrow Transplant. 2019;25:301–6.
27. Desai N, Altareb M, Remberger M, Chen C, Alfaro Moya T, Al-Shaibani E, et al. PTCy-based graft-versus-host disease prophylaxis for matched sibling donor allogeneic hematopoietic cell transplantation. Blood Adv. 2025;9:660–9.
28. Zu Y, Gui R, Li Z, Wang J, Zhang Y, Yu F, et al. Low-dose PTCy plus low-dose ATG as GVHD prophylaxis after UD-PBSCT for hematologic malignancies: a prospective, multicenter, randomized controlled trial. Blood Cancer J. 2023;13:10.
29. Huang R, Chen T, Wang S, Wang J, Su Y, Liu J, et al. Mesenchymal stem cells for prophylaxis of chronic graft-vs-host disease after Haploidentical hematopoietic stem cell transplant: an open-label randomized clinical trial. JAMA Oncol. 2024;10:220–6.
30. Wu H, Shi J, Luo Y, Yu J, Fu H, Liu L, et al. A multicenter randomized controlled trial of low-dose ruxolitinib for Gvhd prophylaxis in haploidentical hematopoietic stem cell transplantation. Blood. 2024;144:1044.
31. Koshy AG, Kim HT, Liegel J, Arnason J, Ho VT, Antin JH, et al. Phase 2 clinical trial evaluating abatacept in patients with steroid-refractory chronic graft-versus-host disease. Blood. 2023;141:2932–43.
32. Chorão P, Villalba M, Balaguer-Roselló A, Montoro J, Granados P, Gilabert C, et al. Incidence, risk factors, and outcomes of BK hemorrhagic cystitis in hematopoietic stem cell transplantation from HLA-matched and haploidentical donors with post-transplant cyclophosphamide. Transplant Cell Ther. 2024;31:182e1–182.e11.

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