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Alemtuzumab, Dual Graft-versus-Host Disease Prophylaxis, and Lower CD3$^+$ T Cell Doses Equalize Rates of Acute and Chronic Graft-versus-Host Disease in Pediatric Patients Receiving Allogeneic Hematopoietic Stem Cell Transplantation with Matched Unrelated Donor Peripheral Blood Stem Cells or Bone Marrow Grafts

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Data comparing hematopoietic stem cell transplantation (HSCT) using bone marrow (BM) or peripheral blood stem cell (PBSC) grafts in children after alemtuzumab-based conditioning are lacking. We investigated whether in vivo T cell depletion using alemtuzumab could reduce the risk of severe acute graft-versus-host disease (aGVHD) and chronic GVHD (cGVHD) after HSCT with matched unrelated donor (MUD) BM or PBSCs. This retrospective multicenter study included 397 children (BM group, n = 202; PBSC group, n = 195) who underwent first MUD HSCT at 9 pediatric centers in the United Kingdom between 2015 and 2019. The median age at transplantation was 7.0 years (range, .1 to 19.3 years), and the median duration of follow-up was 3.1 years (range, .3 to 7.5 years). The 3-year overall

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survival was 81% for the entire cohort (BM group, 80%; PBSC group, 81%). The incidence of grade II-IV aGVHD was significantly higher in the PBSC group (31%) compared to the BM group (31% versus 19%; $P = .003$), with no difference in the incidence of grade III-IV aGVHD (BM, 7%; PBSC, 12%; $P = .17$). $CD3^+$ T cell dose $>5 \times 10^8$/kg and the use of PBSCs were independent predictors of grade II-IV aGVHD. When considering $CD3^+$ T cell dose and GVHD prophylaxis, PBSC transplantation with a calcineurin inhibitor (CNI) and mycophenolate mofetil (MMF) and a $CD3^+$ T cell dose $\leq 5 \times 10^8$/kg had a comparable grade II-IV aGVHD to BM transplantation plus a CNI (20% versus 18%; $P = .52$). PBSC transplantation was associated with a lower incidence of cGVHD compared to BM transplantation (6% versus 11%; $P = .03$). Within the limits of this study, we identified a potential strategy to reduce the risk of severe GVHD in pediatric PBSC recipients that includes a combination of in vivo T cell depletion using alemtuzumab and dual GVHD prophylaxis (with a CNI and MMF) and limiting the $CD3^+$ T cell dose to $\leq 5 \times 10^8$/kg.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is a therapeutic option for malignant disorders and an increasing number of nonmalignant disorders. Bone marrow (BM) was the sole source of hematopoietic stem cells for many decades until the use of peripheral blood stem cells (PBSCs) was introduced into the clinical setting after successful mobilization of PBSCs using granulocyte colony-stimulating factor [1–4]. Although PBSC grafts have largely replaced BM grafts for HSCT in adults for faster engraftment and lower transplantation-related mortality (TRM), BM remains the preferred stem cell source in children. The use of PBSCs historically has been associated with higher rates of graft-versus-host disease (GVHD), both acute (aGVHD) and chronic (cGVHD), possibly attributed to the higher T cell content in the grafts [5,6]. Although the use of PBSC grafts is one of the major advances in transplantation medicine, serotherapy also has revolutionized HSCT strategies, particularly for unrelated donor transplantation. Antithymocyte globulin (ATG) and alemtuzumab, widely used serotherapies in HSCT, are associated with reduced GVHD and improved outcomes, such that results of matched unrelated donor (MUD) HSCT approach those of matched related donor HSCT.

Alemtuzumab is a humanized IgG monoclonal antibody that targets the CD52 antigen, a membrane protein expressed on the surface of peripheral blood immune cells and particularly on T cells, but not on hematopoietic stem cells. Alemtuzumab was developed in 1979 at the Pathology Department of the University of Cambridge, anticipating that infusion prior to HSCT would spare stem cells but eliminate donor T cells via complement-dependent cell lysis and/or antibody-dependent cell-mediated cytotoxicity and thereby prevent GVHD [7]. In the United Kingdom, alemtuzumab has been commonly used for in vivo T cell depletion in children since the 1980s, but knowledge of its impact on PBSCs versus BM as the stem cell source in children is lacking. Because PBSC grafts contain a higher stem cell dose for cryopreservation, this retrospective study was designed during the Coronavirus disease 2019 pandemic to explore the impact of alemtuzumab on PBSC versus BM MUD HSCT in children.

PATIENTS AND METHODS

This retrospective study recruited children and adolescents who underwent their first MUD (10/10 HLA-matched) HSCT using alemtuzumab-based conditioning and unmanipulated PBSC or BM grafts at 9 pediatric transplantation centers in the United Kingdom between January 2015 and December 2019. The participating centers were the Great North Children's Hospital, Royal Manchester Children's Hospital, Great Ormond Street Hospital for Children, Leeds Children's Hospital, Glasgow Children's Hospital, Sheffield Children's Hospital, Royal Marsden Hospital, Bristol Children's Hospital, and University College Hospital London. Clinical and laboratory data were retrieved from the study centers' transplantation databases and medical records; the questionnaire on GVHD is summarized in Supplementary Data. A total of 403 patients were eligible for the study during the study period, 6 of whom were excluded because of concurrent use of ATG (n = 1) or ex vivo T cell depletion using alemtuzumab (n = 5). All patients received i.v. alemtuzumab,

with the timing of alemtuzumab treatment differing among centers.

Definition and Endpoints

The primary endpoints were aGVHD and cGVHD. The secondary endpoints were overall survival (OS), graft failure, TRM, and relapse-related mortality (RRM). TRM was defined as death due to any transplantation-related cause other than disease relapse, and RRM was defined as death due to relapse of malignancy. For the purpose of this analysis, conditioning regimen intensity was classified as myeloablative conditioning (MAC), reduced-toxicity conditioning (RTC), or reduced-intensity conditioning (RIC). MAC referred to total body irradiation (TBI; >8 Gy) with cyclophosphamide (TBI-Cy) or etoposide (TBI-VP16), busulfan (area under the curve [AUC] >65 mg/Lxh)-cyclophosphamide (BuCy) with or without melphalan (BuCyMel), and treosulfan-cyclophosphamide (TreoCy). RTC regimens included pharmacokinetic-targeted busulfan (AUC >65 mg/Lxh)-fludarabine (BuFlu) and fludarabine-treosulfan-thiotepa (FluTreoThio). RIC regimens included treosulfan-fludarabine (TreoFlu), fludarabine-melphalan (FluMel), pharmacokinetic-targeted busulfan (AUC <65 mg/Lxh)-fludarabine (BuFlu), and fludarabine-cyclophosphamide (FluCy).

Statistical Analysis

Quantitative variables were described as median and range, and categorical variables were reported as count and percentage. Competing risks methods were used for the cumulative incidence (CIN) of aGVHD and cGVHD, with death, graft failure, and GVHD after donor lymphocyte infusion (DLI) treated as competing events. Subgroup differences in aGVHD and cGVHD were evaluated by Gray's test. The Fine-Gray model was used to identify independent predictors of GVHD. Subgroup differences in OS were evaluated by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model for OS to identify independent factors that are prognostic of OS. Covariates considered in model building were the indication for HSCT (malignant versus nonmalignant disorders), conditioning (MAC versus RTC/RIC), GVHD prophylaxis (calcineurin inhibitor [CNI] monotherapy versus CNI + mycophenolate mofetil [MMF] versus CNI + methotrexate [MTX]), stem cell source (BM versus PBSC), and $CD3^+$ T cell dose ($\leq 5 \times 10^8$/kg versus $>5 \times 10^8$/kg versus nonevaluated). All variables with a P value <.25 in univariate analysis were included in a multivariate analysis. All estimates were reported with 95% confidence intervals (CI).

In the subgroup analyses, we assessed whether $CD3^+$ T cell dose and GVHD prophylaxis had any impact on GVHD risk. The stem cell source was stratified according to $CD3^+$ T cell dose and GVHD prophylaxis as (1) BM + CNI (n = 149); (2) BM + CNI + MMF (n = 29); (3) BM + CNI + MTX (n = 24); (4) PBSC + CNI + MMF + $CD3^+$ $\leq 5 \times 10^8$/kg (n = 96); (5) PBSC + CNI + MMF + $CD3^+$ $>5 \times 10^8$/kg (n = 42); (6) PBSC + CNI + MMF + $CD3^+$ nonevaluated (n = 21); (7) PBSC + CNI + $CD3^+$ $\leq 5 \times 10^8$/kg (n = 14); and (8) PBSC + CNI + $CD3^+$ nonevaluated (n = 32). BM was not stratified according to $CD3^+$ cell dose, given that the median $CD3^+$ T cell dose was $.64 \times 10^8$/kg, and only 11 BM recipients (5%) were known to have received a $CD3^+$ T cell dose $>5 \times 10^8$/kg.

Data Sharing

For original data, please contact nshl5@newcastle.ac.uk.

RESULTS

Patient and Transplantation Characteristics

Patient and transplantation characteristics are summarized in Table 1. Of the 397 evaluable patients, 202 (51%) underwent BM HSCT and 195 (49%) underwent PBSC HSCT. The median age at HSCT was 7.0 years (range, .1 to 19.3 years) for the entire cohort. The PBSC group was significantly younger than the BM group (median age, 6.3 years [range, .01 to 19.3 years] versus 8.0 years [range, .3 to 18.8 years]; P = .02). The diagnoses were malignancy in 164 patients (41%; acute lymphoblastic leukemia, n = 104; acute myeloid leukemia, n = 27; biphenotypical leukemia, n = 5; myelodysplastic syndrome, n = 13; juvenile myelomonocytic leukemia, n = 2; lymphoma, n = 10; others, n = 3) and nonmalignant disorders in 233 patients (59%; severe combined immunodeficiency [SCID], n = 7; non-SCID inborn errors of immunity, n = 118; aplastic anemia, n = 45; metabolic disorders, n = 24; bone marrow failure, n = 21; hemophagocytic lymphohistiocytosis, n = 12; others, n = 6). A larger proportion of PBSC HSCT recipients than BM HSCT recipients underwent transplantation (67% [n = 131] versus 51% [n = 102]; P = .001).

The dose of alemtuzumab was .9 to 1.0 mg/kg in 383 patients (96%), .8 mg/kg in 2 (.5%), and between .3 and .5 mg/kg in the remaining 13 (3.5%). There was a significant difference in the

Table 1
Transplantation Characteristics and Outcomes According to Stem Cell Source

Characteristic	All	BM Group	PBSC Group	*P* Value
Number of patients	397	202	195	
Age at transplantation, yr, median (range)	7.0 (1.0-19.3)	8.0 (.3-18.8)	6.3 (.1-19.3)	.02
Diagnosis, n (%)				.001
Malignant disorders*	164 (41)	100 (49)	64 (33)	
Nonmalignant disorders†	233 (58)	102 (51)	131 (67)	
Conditioning regimen, n (%)				<.001
MAC	137 (35)	87 (43)	50 (26)	
RTC	91 (23)	59 (29)	32 (17)	
RIC	169 (42)	58 (28)	111 (57)	
GVHD prophylaxis, n (%)				
CNI alone	197 (50)	149 (73)	48 (24)	<.001
CNI + MMF	169 (43)	29 (14)	140 (72)	
CNI + MTX	31 (9)	24 (11)	7 (4)	
Stem cell dose				
TNC dose, $\times 10^8$/kg, median (range)	8.0 (.8-35.8)	4.5 (.8-25.3)	12.1 (1.1-35.8)	<.001
$CD34^+$ T cell dose, $\times 10^6$/kg, median (range)	8.4 (.52-59.7)	5.1 (.5-30.2)	10.6 (1.0 - 59.7)	<.001
$CD3^+$ T cell dose, $\times 10^8$/kg, median (range)	3.5 (.03-10.0)	.64 (.03-10.0)	4.8 (.27-10.0)	<.001
Nonevaluated $CD3^+$ T cell dose, n (%)	171 (43)	115 (56)	57 (30)	<.001
Engraftment kinetics				
Time to neutrophil engraftment, d, median (range)	17 (8-103)	20 (9-103)	16 (8-42)	<.001
Time to platelet engraftment, d, median (range)	16 (4-370)	21 (4-370)	15 (4-104)	<.001
Graft failure, n (%)	22 (6)	14 (7)	8 (4)	.22
Whole blood chimerism, n (%)				
Full donor chimerism (≥95%)	215 (77)	112 (79)	103 (75)	.53
Mixed donor chimerism (<95%)	63 (23)	30 (21)	33 (24)	

TNC indicates total nucleated cells.
* Malignant disorders: acute lymphoblastic leukemia, n = 104; acute myeloid leukemia, n = 27; biphenotypical leukemia, n = 5; myelodysplastic syndrome, n = 13; juvenile myelomonocytic leukemia, n = 2; lymphoma, n = 10; others, n = 3.
† Nonmalignant disorders: severe combined immunodeficiency (SCID), n = 7; non-SCID inborn errors of immunity, n = 118; aplastic anemia, n = 45; metabolic disorders, n = 24; BM failure, n = 21; hemophagocytic lymphohistiocytosis, n = 12; others, n = 6.

conditioning regimen intensity between the PBSC and BM groups ($P < .001$). Myeloablative conditioning (MAC) was used in 43% (n = 87) of BM HSCT recipients, compared to 26% (n = 51) of PBSC HSCT recipients, whereas reduced-toxicity conditioning was used in 29% (n = 59) of PBSC HSCT recipients and in 17% (n = 32) of BM HSCT recipients. The use of reduced-intensity conditioning (RIC) was more frequent in the PBSC group (57% [n = 110] versus 28% [n = 58]). The most frequently used GVHD prophylaxis regimens were monotherapy with a calcineurin inhibitor (CNI) in 73% (n = 149; cyclosporine [CsA], n = 144; tacrolimus, n = 5) of BM HSCT recipients and dual GVHD prophylaxis with CNI + MMF in 72% (n = 140) of PBSC recipients ($P < .001$).

The median total nucleated cell dose was significantly higher in the PBSC group (12.1×10^8/kg; range, 1.1 to 35.8×10^8/kg) compared to the BM group (4.5×10^8/kg; range, .8 to 25.3×10^8/kg; $P < .001$) (Supplementary Figure S1). The median $CD34^+$ cell dose was significantly higher in the PBSC group (10.6×10^6/kg [range, 1.0 to 59.7×10^6/kg] versus 5.1×10^6/kg [range, .5 to 30.2×10^6/kg]; $P < .001$). The $CD3^+$ T cell dose was evaluated in 226 patients (57%; PBSC, 71% [n = 138]; BM, 44% [n = 88]), revealing a significantly higher $CD3^+$ T cell dose in the PBSC group (4.8×10^8/kg [range, .27 to 244.64×10^8/kg] versus $.64 \times 10^8$/kg [range, .03 to 10×10^8/kg].

Engraftment Kinetics and Graft Failure

Neutrophil and platelet engraftment were significantly faster in the PBSC group compared with the BM group. The median time to neutrophil engraftment was 16 days (range, 8 to 42 days) after PBSC HSCT and 20 days (range, 9 to 103 days) after BM HSCT ($P < .001$). The median time

to platelet engraftment in the 2 groups was 15 days (range, 4 to 104 days) and 21 days (range, 4 to 370 days), respectively ($P < .001$).

Graft failure occurred in 6% of the study population (n = 22). There was no significant difference between the 2 groups in the proportion of patients who developed graft failure (BM, 7% [n = 14]; PBSC, 4% [n = 8]; $P = .22$). In the BM group, types of graft failure included primary aplasia (n = 4), secondary aplasia (n = 1), and secondary reconstitution (n = 9). In the PBSC, types of graft failure were primary aplasia (n = 1), secondary aplasia (n = 1), primary reconstitution (n = 2), and secondary reconstitution (n = 4).

GVHD

Of the 10 patients who received DLI, 6 did not have aGVHD before or after DLI, 2 had grade II aGVHD that resolved before DLI, 1 developed cGVHD after DLI, 1 did not have GVHD prior to DLI but developed grade II GVHD and cGVHD after DLI, and 1 had grade I GVHD after DLI but underwent second HSCT for relapsed biphenotypical leukemia. Considering GVHD after DLI, graft failure, and death as competing events, the CIN of grade II-IV and grade III-IV aGVHD at day 100 post-HSCT for the entire cohort was 23% (95% CI, 18% to 29%) and 10% (95% CI, 6% to 13%), respectively. PBSC HSCT was associated with a higher CIN of grade II-IV aGVHD (31% [95% CI, 23% to 42%] versus 19% [95% CI, 23% to 42%]; $P = .003$; Figure 1A) but there was no significant difference in grade III-IV aGVHD (BM, 7% [95% CI, 4% to 13%]; PBSC, 12% [95% CI, 8% to 20%]; $P = .17$). On univariate analysis for grade II-IV aGVHD, transplantation for malignancy, MAC, PBSC graft, and a $CD3^+$ T cell dose $>5 \times 10^8$/kg were significantly associated with a higher CIN of grade II-IV aGVHD (Table 2). On multivariate analysis, PBSC (sub-hazard ratio [SHR], 2.16; 95% CI, 1.38 to 3.37; $P = .001$) and $CD3^+$ T cell dose $>5 \times 10^8$/kg (SHR, 2.73; 95% CI, 1.51 to 4.94; $P = .001$) were independently associated with a higher CIN of grade II-IV aGVHD (Table 3). Univariate analysis identified malignant disorders and MAC as associated with a higher CIN of grade III-IV aGVHD, but neither indication nor conditioning was independently associated with grade III-IV aGVHD on multivariate analysis.

The CIR of cGVHD at 1 year after HSCT was 9% (95% CI, 6% to 12%) for the entire cohort. Of 36 patients with cGVHD, 22 (61%) had progressed from aGVHD, 4 (11%) developed cGVHD following a period of aGVHD resolution, and 10 (28%) had de novo cGVHD. The severity was limited in 23 patients (64%; BM, n = 16; PBSC, n = 7) and extensive in 13 (36%; BM, n = 9; PBSC, n = 4). In contrast to aGVHD, PBSC HSCT was associated with a lower CIN of cGVHD (6%; 95% CI, 3% to 10%) compared to BM HSCT (11%; 95% CI, 7% to 17%; $P = .03$; Figure 1D). Other significant predictors on univariate analysis were transplantation for malignancy, MAC, and $CD3^+$ T cell dose. On multivariate analysis,

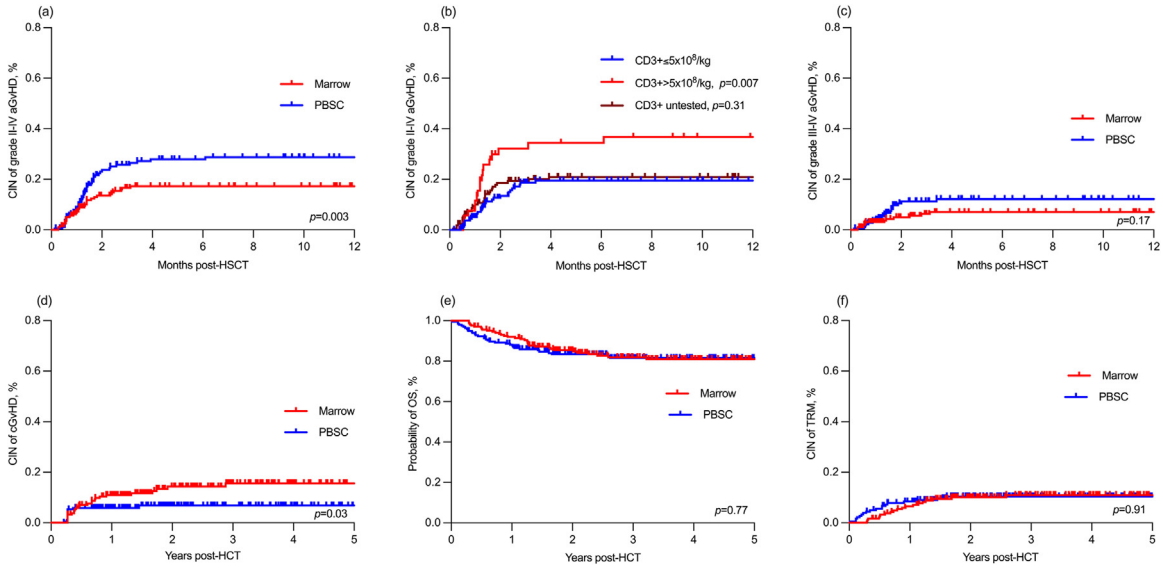

Figure 1. GVHD, OS, and TRM according to stem cell source and graft $CD3^+$ T cell dose. The rate of grade II-IV aGVHD was significantly higher in the PBSC group compared to the BM group (A) and in patients who received $CD3^+$ T cell dose $>5 \times 10^8$/kg (B). The incidence of grade III-IV aGVHD was comparable in the 2 groups (C). The incidence of cGVHD was significantly higher in the BM group (D). The stem cell source had no significant impact on OS (E) or TRM (F).

Table 2
Univariate Analysis for aGVHD and cGVHD

Variable	Grade II-IV aGVHD			Grade III-IV aGVHD			cGVHD		
	Day 100 CIN, % (95% CI)	SHR (95% CI)	*P*	Day 100 CIN, % (95% CI)	SHR (95% CI)	*P*	1-yr CIN, % (95% CI)	SHR (95% CI)	*P*
Diagnosis									
Nonmalignant (n = 233)	18 (13-25)	1		7 (4-12)	1		5 (3-9)	1	
Malignant (n = 164)	33 (26-46)	2.08 (1.36-3.19)	**.001**	14 (9-23)	2.14 (1.05-4.37)	**.04**	13 (6-21)	2.32 (1.20-4.49)	**.01**
Conditioning									
MAC (n = 138)	41 (30-56)			15 (9-25)			16 (10-25)	1	
RTC/RIC (n = 259)	17 (13-24)	.45 (.29-.68)	<**.001**	7 (4-12)	1 (.22-9.94)	**.03**	5 (3-9)	.41 (.21-.79)	**.007**
GVHD prophylaxis									
CNI (n = 197)	21 (15-30)	1		8 (5-14)	1		11 (9-22)	1	
CNI + MMF (n = 169)	28 (21-39)	1.22 (.79-1.89)	.37	11 (6-18)	1.21 (.57-2.58)	.61	5 (3-10)	.52 (.25-1.09)	**.09**
CNI + MTX (n = 31)	25 (11-57)	.99 (.42-2.37)	.99	13 (4-39)	1.47 (.42-5.12)	.55	25 (4-37)	.82 (.25-2.69)	.74
Stem cell source									
BM (n = 202)	19 (13-27)	1		7 (4-13)	1		11 (7-17)	1	
PBSC (n = 195)	31 (23-42)	2.94 (1.24-3.03)	**.003**	12 (8-20)	1.66 (.80-3.45)	.17	6 (3-10)	.46 (.23-.94)	**.03**
$CD3^+$ T cell dose									
$\leq 5 \times 10^8$/kg (n = 168)*	21 (15-31)	1		8 (5-15)	1		5 (2-10)	1	
$>5 \times 10^8$/kg (n = 58)†	41 (25-66)	2.19 (1.25-3.87)	**.007**	14 (6-32)	1.78 (.25-1.21)	.25	10 (4-23)	2.06 (.71-5.97)	.18
Nonevaluated (n = 171)‡	23 (16-33)	1.28 (.79-2.08)	.31	10 (6-17)	1.21 (.55-2.70)	.64	12 (7-18)	2.69 (1.21-6.00)	**.02**

* $CD3^+ \leq 5 \times 10^8$ /kg: BM, n = 77; PBSC, n = 9.
† $CD3^+ > 5 \times 10^8$ /kg: BM, n = 11; PBSC, n = 47.
‡ $CD3^+$ nonevaluated: BM, n = 114; PBSC, n = 57.

Table 3
Multivariate Analysis for aGVHD and cGVHD

Variable	Grade II-IV aGVHD		Grade III-IV aGVHD		cGVHD	
	SHR (95% CI)	*P*	SHR (95% CI)	*P*	SHR (95% CI)	*P*
Malignant versus nonmalignant disorders	1.52 (.53-4.34)	.43	1.99 (.29-13.4)	.63	1.44 (.41-5.57)	.54
MAC versus RTC/RIC	.42 (.15-1.18)	.10	.59 (.09-3.99)	.55	.57 (.16-2.01)	.38
PBSC versus BM	2.16 (1.38-3.37)	.001	1.88 (.91-3.88)	.09	.47 (.22-1.01)	.052
CD3 $>5 \times 10^8$/kg versus $CD3^+ \leq 5 \times 10^8$/kg	2.73 (1.51-4.94)	.001	2.25 (.77-6.62)	.14	3.58 (1.19-10.7)	.02
$CD3^+$ nonevaluated versus $CD3^+ \leq 5 \times 10^8$/kg	1.02 (.63-1.65)	.94	.96 (.44-2.11)	.93	2.06 (.94-4.54)	.07

$CD3^+$ T cell dose $>5 \times 10^8$/kg independently influenced cGVHD (SHR, 3.56; 95% CI, 1.19 to 10.69; $P = .02$).

In the subgroup analysis for stratification of stem cell source by $CD3^+$ T cell dose and GVHD prophylaxis, PBSC HSCT with CNI+MMF and $CD3^+$ T cell dose $\leq 5 \times 10^8$/kg and BM HSCT with CNI monotherapy had a comparable CIN of grade II-IV aGVHD (20% [95% CI, 11% to 33%] versus 18% [95% CI, 12% to 18%]; $P = .52$) (Table 4, Figure 2). PBSC HSCT with CNI+MMF and $CD3^+$ T cell dose $>5 \times 10^8$/kg (CIN, 51% [95% CI, 30% to 86%]; SHR, 3.22 [95% CI, 1.74 to 5.96]; $P < .001$) and PBSC HSCT with CNI monotherapy with nonevaluated $CD3^+$ T cell dose (CIN, 32%; 95% CI, 10% to 100%; $P = .001$) were significantly associated with a higher CIN of grade II-IV aGVHD (Figure 2). For grade III-IV aGVHD, none of these combinations were associated with severe aGVHD. For cGVHD, none of the PBSC recipients with a CD3 T cell dose $\leq 5 \times 10^8$/kg with either CNI monotherapy ($P < .001$) or CNI+MMF ($P < .001$) developed cGVHD, compared with a CIN of 12% (95% CI, 8% to 20%) after BM HSCT with CNI monotherapy. Only 2 PBSC HSCT recipients received CNI monotherapy and a $CD3^+$ dose $>5 \times 10^8$/kg, and both developed aGVHD (1 with grade I and 1 with grade II). Seven PBSC HSCT recipients received CNI+MTX ($CD3^+$ dose $\leq 5 \times 10^8$/kg, n = 1; $CD3^+$ dose $>5 \times 10^8$/kg, n = 2; undetermined $CD3^+$ dose, n = 4), 1 of whom had grade IV aGVHD.

OS and TRM

The median duration of follow-up of surviving patients was 3.1 years (range, .3 to 7.5 years). OS at 3 years post-HSCT was 81% (95% CI, 77% to 85%) for the entire cohort, 80% (95% CI, 73% to 86%) for the BM group, and 81% (95% CI, 74% to 89%) for the PBSC group ($P = .69$). OS by disease type was 69% (95% CI, 61% to 76%) for malignant disorders and 90% (95% CI, 85% to 93%) for nonmalignant disorders ($P < .001$) (Table 5). In the malignant disease subcohort, OS was 68% (95% CI, 59% to 76%) after MAC (n = 137) and 67% (95% CI, 44% to 82%) after RTC/RIC (n = 27; $P = .80$). In the nonmalignant disease subcohort, no patients received MAC, 73 patients (31%) received RTC, and 160 (69%) received RIC. The 3-year OS was 94% (95% CI, 85% to 93%) after RTC and 89% (95% CI, 82% to 93%) after RIC. GVHD prophylaxis and $CD3^+$ T cell dose had no association with OS.

The CIN of TRM at 1 year post-HSCT was 7% (95% CI, 5% to 11%) for the entire cohort, 10% in the BM group and 8% in the PBSC group ($P = .91$). On univariate analysis, the use of CNI+MTX was significantly associated with higher TRM (29% [95% CI, 13% to 61%]; SHR, 3.36 [95% CI, 1.38 to 8.19]; $P = .008$) compared to CNI (8%; 95% CI, 5% to 14%) and CNI+MMF (11% [95% CI, 6% to 18%]; SHR, 1.29; [95% CI, .62 to 2.68]; $P = .50$). The indication for transplantation, conditioning regimen, stem cell source ($P = .91$; Figure 1E), and $CD3^+$ cell dose ($P = 3.38$) had no impact on TRM.

In the 37 deceased patients, the causes of TRM were infection in 23 patients (61%; including sepsis in 3, cytomegalovirus in 3, adenovirus in 3, adenovirus and fungal infection in 2, cytomegalovirus and fungal infection in 1, influenza A virus in 1, parainfluenza virus in 1, fungal infection in 4, toxoplasmosis in 2, pneumococcus in 1, and unspecified infection in 2), respiratory failure in 5 patients, multiorgan failure in 3, thrombotic microangiopathy in 1, cGVHD in 1 (BM recipient), encephalopathy in 1, cardiac failure in 1, and unspecified cause in 2.

In the malignant disease subcohort, when considering TRM as a competing event, the 1-year CIN of relapse was 29% (95% CI, 21% to 39%) and that of RRM was 16% (95% CI, 11% to 24%). Stem cell source had no impact on the CIN of relapse (BM: 27% [95% CI, 18% to 42%]; PBSC: 30% [95% CI, 18% to 50%; $P = .94$) or RRM (BM: 14% [95% CI, 8% to 25%]; PBSC: 19% [95% CI, 8% to 25%; $P = .98$).

Donor Chimerism

Latest donor chimerism data were available for 278 patients (70%) who were alive and engrafted

Table 4
Impact of GVHD Prophylaxis and $CD3^+$ T Cell Dose on PBSC and BM HSCT Recipients

Variable	No.	Grade II-IV aGVHD			Grade III-IV aGVHD			cGVHD		
		Day +100 CIN, % (95% CI)	SHR (95% CI)	*P*	Day +100 CIN, % (95% CI)	SHR (95% CI)	*P*	1-year CIN, % (95% CI)	SHR (95% CI)	*P*
BM, CNI	149	18 (12-28)	1		7 (4-14)	1		12 (8-20)	1	
BM, CNI + MMF	29	13 (4-42)	.63 (.19-2.11)	.46	5 (1-37)	.58 (.98- 4.47)	.60	7 (2-28)	.69 (.21-2.23)	.53
BM, CNI + MTX	24	29 (12-70)	1.56 (.59-4.14)	.37	11 (3-45)	1.55 (.33-7.16)	.57	10 (3-40)	.65 (.15-2.7)	.55
PBSC, CNI + MMF, $CD3^+ \leq 5 \times 10^8$/kg	96	20 (11-33)	1.23 (.65-2.38)	.52	9 (3-17)	1.24 (.44-3.46)	.69	0	0	**<.001**
PBSC, CNI + MMF, $CD3^+ > 5 \times 10^8$/kg	43	51 (30-86)	3.22 (1.74-5.96)	**<.001**	16 (6-39)	2.23 (.76-6.86)	.14	9 (3-28)	.50 (.15-1.70)	.269
PBSC, CNI + MMF, $CD3^+$ nonevaluated	21	27 (10-74)	1.69 (.65-4.38)	.28	12 (3-52)	1.77 (.38-8.37)	.47	22 (8-58)	1.50 (.49-4.55)	.47
PBSC, CNI, $CD3^+ \leq 5 \times 10^8$/kg	14	32 (10-100)	1.57 (.46-5.40)	.47	10 (1-71)	1.43 (.18-11.3)	.34	0	0	**<.001**
PBSC, CNI, $CD3^+$ nonevaluated	32	29 (13-67)	3.01 (1.54-5.87)	**.001**	17 (5-54)	2.01 (.56-7.54)	.28	9 (3-29)	.69 (.20-2.37)	.56

after their first MUD HSCT. There was no significant difference in median whole blood chimerism between BM recipients (n =143; median; 100%; 95% CI, 6% to 100%) and PBSC recipients (n = 136; median, 100%; 95% CI, 4% to 100%) (P = .88). The proportion of patients with mixed chimerism (<95%) was comparable in the BM and PBSC groups (21% versus 24%; P = .53).

DISCUSSION

The present retrospective study is the first multicenter analysis comparing BM HSCT and PBSC HSCT in children receiving alemtuzumab-based conditioning. Several important observations emerge from this analysis. The use of PBSCs was associated with faster neutrophil and platelet engraftment but did not have any significant impact on graft failure and donor chimerism. There was no significant survival difference between BM recipients and PBSC recipients after MUD HSCT using alemtuzumab-based conditioning. TRM also was comparable in the BM and PBSC recipients. In the malignant disease subcohort, stem cell source had no significant impact on relapse (BM, 27%; PBSC, 30%) or RRM (BM, 14%; PBSC, 19%). Although the rate of grade II-IV aGVHD was higher in the PBSC recipients, the rate of grade III-IV aGVHD was comparable in the 2 groups. The cGVHD rate was low at 9% for the entire cohort but was higher in BM recipients compared to PBSC recipients, which might be explained by the use of dual GVHD prophylaxis with CNI+MMF in the majority of PBSC recipients. Nevertheless, in patients with a known graft $CD3^+$ cell dose, a higher $CD3^+$ T cell dose was significantly associated with the occurrence of aGVHD and cGVHD in children undergoing MUD HSCT with alemtuzumab-based conditioning. The rate of grade II-IV aGVHD was comparable in recipients of PBSCs with CNI+MMF and a $CD3^+$ T cell dose $\leq 5 \times 10^8$/kg and recipients of BM + CNI monotherapy, which is a common practice in pediatric HSCT in the United Kingdom.

The use of PBSCs in pediatric patients often has been associated with an increased risk of aGVHD and cGVHD. Eapen et al. [8] reported that in 773 pediatric recipients of HLA-matched sibling donor (MSD) HSCT (BM, n = 603; PBSCs, n = 143) for acute leukemia between 1995 and 2000, the risk of grade II-IV aGVHD was similar in the 2 groups but rates of TRM, treatment failure, and cGVHD were higher in the PBSC group. No patients received serotherapy, and GVHD prophylaxis was mainly CsA with/without MTX. In a recent similar study reported by Keesler et al. [9] of pediatric

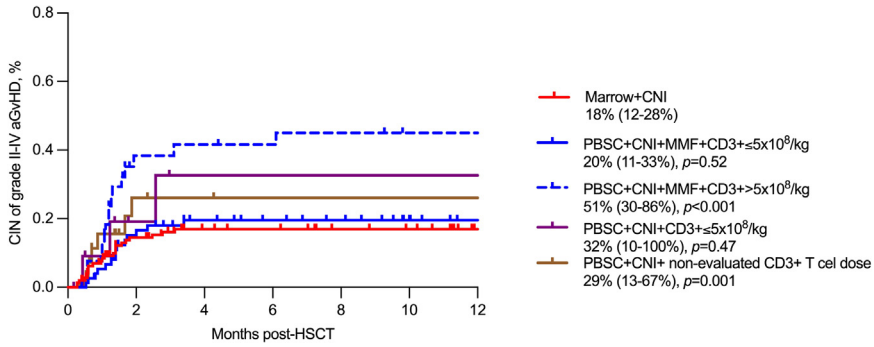

Figure 2. Grade II-IV aGVHD in the BM and PBSC groups, stratified by GVHD prophylaxis and graft $CD3^+$ T cell dose. PBSC with CNI and MMF and a $CD3^+$ T cell dose $\leq 5 \times 10^8$/kg had a comparable grade II-IV aGVHD to BM plus CNI. PBSC plus CNI/MMF plus $CD3^+$ T cell dose $>5 \times 10^8$/kg and PBSC plus CNI plus $CD3^+$ dose nonevaluated had a significantly higher CIN of grade II-IV aGVHD compared to BM plus CNI.

leukemia patients undergoing BM HSCT (n = 650) or PBSC HSCT (n = 222) from an unrelated donor between 2000 and 2012, PBSC HSCT was associated with higher rates of grade II-IV aGVHD, grade III-IV aGVHD, and cGVHD. In that cohort, the risk of relapse was lower after PBSC HSCT, but rates of TRM and overall mortality were higher in PBSC HSCT than in BM HSCT. CNI+MTX was the predominant GVHD prophylaxis regimen in both the BM and PBSC recipients, and in vivo T cell depletion was used in 40% of BM HSCTs and in 38% of PBSC HSCTs [9]. In a study by the European Society for Blood and Marrow Transplantation including 2584 pediatric patients who underwent HSCT for acute lymphoblastic leukemia between 2003 and 2012, TRM and cGVHD were significantly higher with PBSCs compared to other stem cell sources, but OS was similar for all stem cell sources [5]. In the prospective ALL-SCT-BFM 2003 study, there were no differences in OS, TRM, aGVHD, or relapse between BM HSCT and PBSC HSCT from MSDs and other matched donors, but cGVHD was higher after PBSC HSCT from MSDs. In that trial, the MSD HSCT recipients received CsA, whereas the MUD HSCT recipients were given ATG and CsA+MTX [10].

Table 5
Univariate Analysis for OS and TRM

Variable	OS			TRM		
	3-yr OS, % (95% CI)	HR (95% CI)	*P*	1-yr TRM, % (95% CI)	SHR (95% CI)	*P*
Diagnosis						
Nonmalignant (n = 233)	90 (85-93)	1		8 (5-13)	1	
Malignant (n = 164)	69 (61-76)	3.33 (1.99-5.59)	**<.001**	13 (9-21)	1.60 (.83-3.08)	.83
Conditioning						
MAC (n = 138)	69 (59-76)	1		12 (9-25)	1	
RTC/RIC (n = 259)	88 (83-91)	.38 (.24-.62)	**<.001**	7 (4-12)	.74 (.38-1.44)	.37
GVHD prophylaxis						
CNI (n = 197)	80 (73-85)	1		8 (5-14)	1	
CNI + MMF (n = 38)	84 (77-89)	.90 (.54-1.51)	.69	11 (6-18)	1.29 (.62-2.68)	.50
CNI + MTX (n = 68)	72 (51-85)	1.61 (.75-3.49)	.22	29 (13-61)	3.36 (1.38-8.19)	**.008**
Stem cell source						
BM (n = 202)	80 (73-86)	1		10 (6-16)	1	
PBSC (n = 195)	81 (74-86)	1.07 (.67-1.73)	.77	8 (5-14)	.96 (.50-1.84)	.91
$CD3^+$ T cell dose						
$CD3^+ \leq 5 \times 10^8$ /kg (n = 168)*	79 (70-85)	1		8 (4-14)	1	
$CD3^+ > 5 \times 10^8$ /kg (n = 58)†	89 (78-95)	.56 (.23-1.34)	.19	9 (4-21)	1.01 (.39-2.60)	.98
$CD3^+$ nonevaluated (n = 171)‡	79 (72-85)	.87 (.63-1.72)	.87	8 (5-15)	.73 (.35-1.49)	.38

There are a number of obvious differences between the foregoing studies and our present study, the most important being the use of in vivo T cell depletion, type of serotherapy (ATG versus alemtuzumab), and type of GVHD prophylaxis (CsA alone versus CsA+MTX versus CsA+MMF) in PBSC HSCT recipients. Therefore, the findings from these studies are not informative for pediatric patients who receive alemtuzumab-based conditioning.

Randomized controlled trials (RCTs) comparing BM and PBSC HSCT have been performed mainly in adult patients. In a meta-analysis including a total of 1521 adults with hematologic malignancies from 9 RCTs that compared BM versus PBSC HSCT from HLA-matched donors between 1991 and 2012, disease-free survival (P = .6) and NRM or TRM (P = .91) were comparable in the 2 groups [11]. The analysis showed faster neutrophil and platelet engraftment with PBSC grafts. Grade II-IV aGVHD was not lower in the BM group (P = .67), but there was a trend toward a lower incidence of grade III-IV aGVHD in BM recipients (P = .07). BM grafts also were associated with lower rates of overall and extensive cGVHD (P = .001). In this analysis, ATG was used alone as serotherapy in 2.7% of participants (n = 41), and none received alemtuzumab.

In the most recently reported RCT involving 551 adults with malignancy using unrelated donors, Anasetti et al. [12] demonstrated comparable survival in BM and PBSC graft recipients, a lower risk of graft failure in PBSC recipients, and a reduced risk of cGVHD in BM recipients. In that trial, ATG was used in 14% of patients (n = 79), and the remaining patients did not receive serotherapy.

The results of RCTs in adults are not readily applicable to children, given the differences in conditioning, serotherapy, and GVHD prophylaxis between adults and children. Several hypotheses have been postulated to explain the biological differences in GVHD between adult and pediatric patients, including different immune profiles in GVHD, greater thymic function in children (which may account for lower rates of cGVHD), and declining thymic function associated with the onset of puberty [13].

Shaw et al. [14] studied the impact of alemtuzumab in 306 adults and children with malignancy who received myeloablative conditioning and reported no graft source-related difference in the rates of grade II-IV aGVHD (BM, 23%; PBSC, 24%) or grade III-IV aGVHD (BM, 4%; PBSC, 5%) and high cGVHD in both the BM (47%) and PBSC (49%) groups [14]. In contrast, the rate of cGVHD was much lower in our study (10%) and significantly lower in our PBSC group than in our BM group (6% versus 11%). This could be explained by differences in alemtuzumab dose and GVHD prophylaxis between the 2 studies. Various doses and scheduling of alemtuzumab were used in the study of Shaw et al., but 96% of children received .9 to 1.0 mg/kg of alemtuzumab in our study, with the precise schedule and dosage regimen differing according to institutional practice. Although CsA with or without MTX was used in the Shaw et al. study, the majority of PBSC recipients in our study received CsA+MMF for GVHD prophylaxis. In a study reported by Ottaviano et al. [15] comparing alemtuzumab (n = 63) and ATG (n = 35) in pediatric PBSC HSCT recipients with nonmalignant disorders, the rate of severe aGVHD was significantly higher in patients receiving ATG compared with those receiving alemtuzumab (26% versus 10%; P = .05). Extensive cGVHD was seen in 12% of ATG recipients and 5% of alemtuzumab recipients. Rates of OS, event-free survival, and viral reactivation were comparable in the 2 serotherapy groups, but alemtuzumab was associated with delayed T cell reconstitution [15].

Biological differences between BM and PBSC include the content of both hematopoietic progenitor cells and T cells [16]. In contrast to total nucleated cell dose and $CD34^+$ cell dose, $CD3^+$ T cell dose is not routinely measured in all transplant centers. Our study shows that $CD3^+$ T cell dose is 10-fold higher in pediatric PBSC HSCT recipients compared to BM HSCT recipients and strongly suggests that a $CD3^+$ cell dose $>5 \times 10^8$/kg is associated with a higher incidence of grade II-IV aGVHD. Numerous studies have attempted to define the threshold of $CD3^+$ T cells for GVHD, but no definite conclusion has been reached, owing to the various conditioning and serotherapy regimens used in clinical practice. In a study reported by Atkinson et al. [17] involving 16 patients with hematologic malignancy and MSD BM HSCT after in vitro T cell depletion with anti-CD2 T cell antibody or anti-CD8 antibody, a T cell dose of $\leq 1 \times 10^5$/kg was associated with minimal or no aGVHD, and a T cell dose $\geq 1 \times 10^6$/kg was associated with significant GVHD (P = .001). All these patients received high-dose chemotherapy with or without total body irradiation and without in vivo T cell depletion. An analysis from the Acute Leukaemia Working Party of the European Society for Blood and Marrow Transplantation showed that a $CD3^+$ T cell dose $>3.47 \times 10^8$/kg or a $CD34^+$ cell dose $>8.25 \times 10^6$/kg produced an increased incidence of grade III-IV aGVHD (20% versus 6% [P = .03] and 18% versus 7% [P = .02],

respectively). There was no impact on cGVHD or survival [18].

Using the database of the Center for International Blood and Marrow Transplant Research (CIBMTR), Saad et al. [19] studied the impact of T cell dose on GVHD risk after HLA-matched PBSC HSCT in 2736 adult patients, but this study excluded in vivo and ex vivo T cell depletion. In univariate analysis, MSD HSCT and high $CD3^+$ T cell dose ($>1.4 \times 10^8$/kg) were associated with a higher CIN of grade II-IV aGVHD compared to MSD HSCT with low $CD3^+$ T cell dose ($<1.4 \times 10^8$/kg) (33% versus 25%; P = .009), and MUD HSCT with high $CD3^+$ T cell dose ($>1.1 \times 10^8$/kg) was associated with a higher CIN of grade II aGVHD (50% vs 40%; P = .009) and of cGVHD (31% versus 23%; P = .02) compared to MUD HSCT with low $CD3^+$ T cell dose. However, neither regimen showed any influence on engraftment, severe aGVHD, NRM, relapse, disease-free survival, or OS. Multivariate analysis showed no correlation between $CD3^+$ T cell dose and aGVHD or cGVHD in either the MSD or MUD HSCT group [19]. Because the aforementioned studies focused on adult patients, $CD3^+$ T cell doses were relatively low compared to the median CD3+ T cell dose of 4.8×10^8/kg in the pediatric PBSC HSCT recipients in our study, likely due to the lower body weight in children. Nevertheless, none of the previous studies defined the $CD3^+$ T cell dose threshold for pediatric patients after in vivo T cell depletion with alemtuzumab.

There are several limitations to our study. Similar to most retrospective studies, our study group was heterogeneous in several respects. Although the majority of patients received a .9 to 1.0 mg/kg dose of alemtuzumab for both BM and PBSC HSCT, the timing and dosage regimen varied among transplant centers. In addition, although the conditioning regimen in this study was been classified as MAC, RTC, or RIC, the combination of conditioning regimen and GVHD prophylaxis varied within each group. Finally, the $CD3^+$ T cell dose was not measured in 171 of the study participants (43%; BM group, n = 11 [57%]; PBSC group, n = 57 [29%]).

CONCLUSION

Our data indicate that alemtuzumab protects children from severe aGVHD and cGVHD in recipients of both BM and PBSC MUD HSCT. We have identified a potential strategy to optimize the use of PBSC grafts in children, including using CsA + MMF and limiting the graft $CD3^+$ T cell dose. Owing to limitations of this retrospective study, a prospective RCT is needed to verify these findings and guide clinical practice, with potential significant impacts on recipients, donors, and the health economics of HSCT in children.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2023.12.005.

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