Effect of stem cell source on long-term chimerism and event-free survival in children with primary immunodeficiency disorders after fludarabine and melphalan conditioning regimen

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Background: Reduced-intensity conditioning (RIC) regimens are increasingly being used in the transplantation of patients with primary immunodeficiency disorders (PIDs), but there are no large studies looking at long-term lineage-specific chimerism.
Objectives: We sought to analyze long-term chimerism and event-free survival in children undergoing transplantation for PIDs using RIC with fludarabine and melphalan (Flu/Melph) and to study the effect of donor type and stem cell source.
Methods: One hundred forty-two children underwent transplantation with RIC by using Flu/Melph and for PIDs by using bone marrow (n = 93) or peripheral blood stem cells (PBSCs; n = 49). Donors were matched unrelated donors (n = 72), mismatched unrelated donors (n = 37), matched sibling donors (n = 14), matched family donors (n = 12), and mismatched family donors (n = 7).
Results: Overall survival at a median follow-up of 7.5 years was 78%, irrespective of stem cell source or donor type. When bone marrow was used as the stem cell source, 26% of patients ended up with very low levels of donor chimerism (<10% donor), especially in the myeloid lineage. Event-free survival in this group was significantly lower compared with that in the rest of the group (25% vs 70%, $P < .001$). With the use of PBSCs, more than 90% of patients achieved complete donor chimerism or high-level mixed chimerism (>50% donor chimerism) in all lineages.
Conclusions: On the basis of our experience, we would suggest that PBSCs should be the stem cell source of choice in children with PIDs undergoing transplantation with Flu/Melph RIC from a matched donor source. This is most likely to ensure sustained high-level donor chimerism. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Primary immunodeficiency disorder, hematopoietic stem cell transplantation, chimerism, lineage specific, reduced intensity*

The use of reduced-intensity conditioning (RIC) has enabled hematopoietic stem cell transplantation (HSCT) in patients with pre-existing comorbidities that would preclude HSCT by using conventional approaches. After several reports of superior short- and long-term survival after RIC for primary immunodeficiency disorders (PIDs),[1] use of RIC for PIDs is now the treatment of choice in many institutions, especially in the presence of organ toxicities. RIC regimens frequently combine fludarabine with another agent, such as melphalan, low-dose busulfan, low-dose thiotepa, or low-dose total body irradiation.[2] Fludarabine/melphalan (Flu/Melph) is perhaps the most frequently used RIC regimen in adults and children. Mixed chimerism (MC) is frequently seen with RIC regimens but is often sufficient to cure many immunodeficiency disorders, although in some cases of non–severe combined immunodeficiencies (SCIDs), very low levels of MC (<10% donor) might be insufficient for cure. Analysis of lineage-specific chimerism might be more informative than whole blood chimerism in predicting secondary graft loss after RIC transplantation.[3]

To overcome the problems of MC and relapse, most RIC regimens in adults use peripheral blood stem cells (PBSCs) wherein increased T-cell and stem cell numbers enhance the alloreactivity of the graft and competitively occupy stem cell niches to ensure complete/high levels of donor chimerism.[4] In contrast, bone marrow (BM) has hitherto been the stem cell source of choice in pediatric HSCT because of concerns about high rates of chronic graft-versus-host disease (GVHD) with PBSCs and lack of demonstration of any survival advantage with PBSCs in the myeloablative setting.[5] No large studies have been published to date addressing the issue of what constitutes the optimal stem cell source in the RIC setting in pediatrics. We present our long-term follow-up of 142 children undergoing transplantation at a single institution with the same RIC regimen (fludarabine and melphalan plus Campath/antithymocyte globulin [ATG]) for immunodeficiency conditions. This is the largest series of pediatric RIC HSCT looking at lineage-specific chimerism and outcomes by donor type and stem cell source.

METHODS

All patients undergoing transplantation at Great Ormond Street Hospital for PIDs between October 1998 and August 2012 and receiving identical RIC

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Abbreviations used
aGVHD: Acute graft-versus-host disease
BM: Bone marrow
CC: Complete chimerism
cGVHD: Chronic graft-versus-host disease
DLI: Donor lymphocyte infusion
Flu/Melph: Fludarabine and melphalan
GVHD: Graft-versus-host disease
HSCT: Hematopoietic stem cell transplantation
MC: Mixed chimerism
MFD: Matched family donor
MMF: Mycophenolate mofetil
mMFD: Mismatched family donor
mMUD: Mismatched unrelated donor
MSD: Matched sibling donor
MUD: Matched unrelated donor
PBSC: Peripheral blood stem cell
PID: Primary immunodeficiency disorder
RIC: Reduced-intensity conditioning
SCID: Severe combined immunodeficiency

(n = 142) with fludarabine, melphalan, and Campath/ATG are included in this study. The median age at HSCT was 3.29 years (range, 0.19-17.7 years). Written informed consent was obtained from patients or parents before the transplantation procedure in all cases, and the reduced-intensity continuing protocol was registered with the local institutional review board (protocol no. 99MH11).

Donors for the 142 transplants were 10/10 matched unrelated donors (MUDs; n = 72), mismatched unrelated donors (mMUDs; n = 37), matched sibling donors (MSDs; n = 14), matched family donors (MFDs; n = 12), and mismatched family donors (mMFDs; n = 7). Of the 37 mMUDs, 35 of 37 were mismatched at 1 antigenic locus (HLA-A mismatch, n = 15; HLA-C mismatch, n = 15; HLA-DQ mismatch, n = 4; HLA-DR mismatch, n = 1), and 2 of 37 were mismatched at 2 antigenic loci (HLA-A and HLA-B mismatch, n = 1; HLA-B and HLA-C mismatch, n = 1). All 7 mMFDs were mismatched at a single antigenic locus. From 1998 to the end of 2001, donors were typed serologically for class I antigens and by using molecular techniques for class II antigens. From 2002 onward, all donors were typed by molecular techniques for class I and class II antigens.

BM was used as the stem cell source in 93 transplantations, and PBSCs were used in 49 transplantations.

Patients' characteristics are detailed in Table I. The median duration of follow-up is 7.5 years (range, 2.7-12 years). Median follow-up for the BM and PBSC groups are 11.2 and 5.2 years, respectively.

Conditioning regimen

All patients received uniform conditioning with 30 mg/m^2 fludarabine from days −7 to −3 and 140 mg/m^2 melphalan on day −2 and serotherapy with either 0.2 mg/kg alemtuzumab from days −8 to −4 (n = 119) or 2.5 mg/kg ATG (rabbit; Genzyme, Cambridge, Mass) from days −2 to +2 (n = 23). ATG was used in transplantations performed before 2001, and alemtuzumab was used in subsequent transplantations. GVHD prophylaxis was with cyclosporine (n = 86) or cyclosporine plus mycophenolate mofetil (MMF; n = 60). MMF was used in all PBSC transplants and in 11 patients who received BM transplants.

Engraftment and chimerism

Lineage-specific chimerism was assayed from CD3$^+$ T cells and CD15$^+$ granulocytes isolated from peripheral blood by using magnetic bead technology on the autoMACS Pro Separator (Miltenyi Biotec, Bergisch Gladbach, Germany). Cell fraction purities were routinely greater than 95%. Alternatively, PBMCs and granulocytes were isolated with Lymphoprep (Robbins Scientific, Sunnyvale, Calif). The PowerPlex 16 System (Promega UK, Southampton, United Kingdom) was used to PCR amplify 16 fluorescence–labeled short tandem repeat loci in these patient samples. These PCR products were run on an AB3130 Genetic Analyzer and analyzed with GeneMapper v4.0 software (Applied Biosystems, Foster City, Calif).

Complete chimerism (CC) is described as greater than 95% donor cells. MC is defined as the presence of more than 5% host-derived cells on more than 1 occasion. This is further categorized into high-level MC (95% to 50% donor chimerism), low-level MC (49% to 10% donor chimerism), or very low-level MC (<10% donor chimerism). Acute graft-versus-host disease (aGVHD) was graded with the method of Przepiorka et al,[6] and chronic graft-versus-host disease (cGVHD) was graded as none, limited, or extensive.

Withdrawal of immunosuppression

In the absence of GVHD, cyclosporine was tapered from 3 months after HSCT and stopped by 6 months. When used, MMF was weaned from day 28 after HSCT and stopped over 3 weeks in the absence of GVHD. On detection of MC, cyclosporine weaning was started immediately and stopped over 2 to 4 weeks depending on the occurrence of GVHD.

Statistics

Groups were compared by using the Fisher exact test with a 2-tailed *P* value, except where numbers were small, in which case the χ^2 test with Yates correction was used (GraphPad Prism 5; GraphPad Software, La Jolla, California). *P* values equal to or less than .05 were considered statistically significant. Kaplan-Meier curves were compared by using the Mantel-Cox log-rank test. Logistic regression was performed with SPSS software (SPSS, Chicago, Ill) to identify determinants of very low-level chimerism at 1 year after transplantation.

RESULTS

Engraftment and chimerism according to stem cell source

Lineage-specific chimerism was analyzed in the BM and PBSC groups at 1, 3, and 6 months and 1 year after HSCT and yearly thereafter, as shown in Fig 1 and in Table II.

Ninety-three HSCTs were performed with BM as the stem cell source, and 49 HSCTs were performed by using PBSCs as the stem cell source. The mean CD34 and CD3 doses for the BM and PBSC groups were 9.8×10^6/kg and 1.8×10^8/kg and 20×10^6/kg and 7.5×10^8/kg, respectively.

One month after HSCT. *BM group.* Ninety (97%) of 93 patients were alive at 1 month after HSCT, and lineage-specific chimerism data were available in 88 patients. Ninety-eight percent of patients engrafted with full donor chimerism in the T-cell and myeloid lineages.

PBSC group. Forty-eight (98%) of 49 patients were alive at 1 month after HSCT. Only 1 patient had very low-level MC, and the others had CC in both lineages.

Six months after HSCT. *BM group.* Eighty (86%) of 93 patients were alive, and lineage-specific chimerism was available in 79 patients. By 6 months after HSCT, MC was more frequent. Although more than 75% of patients maintained CC or high-level MC in both lineages; chimerism in the myeloid lineage decreased significantly, with 12 (15%) of 79 patients having very low-level MC ($P < .0001$).

PBSC group. Forty-five (92%) of 49 patients were alive. More than 90% of patients maintained CC or high-level MC in both lineages.

TABLE I. Patients' characteristics (n = 142)

Patient details	n (%)	BM	PBSC
Diagnosis			
PID	142 (100%)	93 (65%)	49 (35%)
CID	37 (26%)	27	10
SCID	32 (22%)	25	7
HLH	25 (18%)	14	11
Phagocytic cell disorders	15 (10%)	8	7
T-cell immunodeficiency	18 (13%)	11	7
WAS	8 (6%)	7	1
XLP	7 (5%)	1	6
Donors and stem cell source		93 (65%)	49 (35%)
MUD	72 (50%)	49	23
mMUD	37 (26%)	17	20
MSD	14 (10%)	13	1
MFD	12 (8%)	8	4
mMFD	7 (5%)	6	1
Median age at transplantation (y)	3.29	2.6	5.3
Median year of transplantation		2002	2008
Male sex	89	57	32
Female sex	53	36	17

CID, Combined immunodeficiency; *HLH*, hemophagocytic lymphohistiocytosis; *WAS*, Wiskott-Aldrich syndrome; *XLP*, X-linked lymphoproliferative disorder.

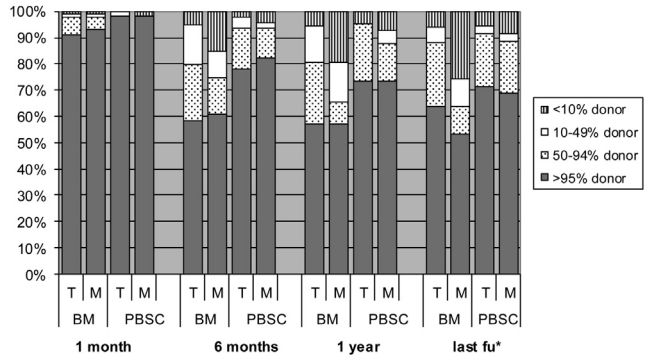

FIG 1. Lineage-specific chimerism in the BM and PBSC groups. Chimerism in the T-cell *(T)* and myeloid lineage *(M)* at 1 month, 6 months, and 1 year after HSCT and at last follow-up in the BM and PBSC groups is shown. At all time points, the incidence of MC was higher in the BM group than in the PBSC group, especially in the myeloid lineage. At last follow-up, the incidence of very low-level MC in the myeloid lineage of the BM group was 26% compared with 8% in the PBSC group. *In case of second transplantation or DLI, chimerism immediately before the second procedure is represented here.

One year after HSCT. *BM group.* Seventy-five (81%) of 93 patients were alive, and lineage-specific chimerism was available in 72. T-cell chimerism remained stable in the majority of patients, but 14 (19%) of 72 patients had very low-level chimerism in the myeloid lineage.

PBSC group. Forty-four (90%) of 49 were alive, and lineage-specific chimerism was available in 41 patients. Once again, the majority of patients maintained stable CC or high-level MC, with only 2 (5%) and 3 (7%) of 41 patients having very low-level T-cell and myeloid chimerism, respectively.

Last follow-up. For the purposes of chimerism studies, last follow-up is defined as the time the patient was last seen and chimerism was analyzed at our institution. This was at an average of 6.9 years after transplantation (range, 0.4-13.1 years) in the BM group and 3.5 years (range, 0.3-9.7 years) in the PBSC group.

BM group. At last follow-up, 71 (76%) of 93 patients were alive, and data were available in 66. T-cell chimerism remained stable between 1 year after transplantation and the last follow-up. There was a further increase in the proportion of patients (17/66 [26%]) with very low-level MC in the myeloid lineage. However, this decrease in myeloid chimerism between 1 year and last follow-up was not statistically significant ($P = .4$).

Seven patients in the BM group with very low-level MC eventually had graft loss with return of disease phenotype, and 6 of them proceeded to a second transplantation procedure. One died without a second procedure. One patient received a donor lymphocyte infusion (DLI) in an attempt to improve chimerism. In patients who underwent a second transplantation procedure, chimerism just before the second procedure is depicted in Fig 1 and Table II.

PBSC group. Forty-one (83%) of 49 patients were alive at last follow-up, and data were available for 35 of them. In the PBSC group there was very little change in T-cell or myeloid chimerism between 1 year and the last follow-up. There was 1 second transplantation procedure in the PBSC group and 3 DLIs.

At last follow-up, there was a higher incidence of very low-level MC in the myeloid series of the BM group (26%) compared with the PBSC group (8%), but this difference was not statistically significant ($P = .41$).

Although donors were typed serologically for class I antigens and by using molecular techniques for class II antigens from 1998-2001 and by using molecular techniques for class I and class II antigens from 2002 onward, there was no difference in the incidence of rejection or very low-level MC in these 2 time periods in either the BM or PBSC groups. In the BM group 8 (20%) of 40 patients experienced rejection or had very low-level MC before 2002 compared with 7 (13%) of 53 after 2002 ($P = .4$). Forty-seven of 49 PBSC transplantations were performed after 2002, and here the incidence of very low-level MC was 2 (4%) of 47. This was not statistically different compared with the incidence of very low-level MC in the BM group after 2002 ($P = .16$). This analysis excluded sibling donors in both time periods.

Chimerism according to donor type at last follow-up

Lineage-specific chimerism was further analyzed according to donor type at last follow-up, as shown in Fig 2 and Table III. Numbers in brackets indicate surviving patients with complete data available.

Matched donors. *BM group.* The majority of patients with MUDs (n = 35) and MFDs (n = 6) had CC or high-level MC in both lineages at last follow-up, with 17% of patients in both these groups achieving very low-level myeloid chimerism. MSDs (n = 10) had a high incidence of very low-level myeloid chimerism (30% [3/10]).

PBSC group. Similarly, the majority of patients receiving MUD (n = 17) and MFD (n = 2) transplants had CC or high-level MC in all lineages. The incidence of very low-level myeloid MC was 18% in the MUD group. Only 1 patient each underwent transplantation with an MFD or MSD, and both have CC or high-level MC in all lineages.

Mismatched donors. *BM group.* Mismatched donors (mMUDs, n = 12; mMFD, n = 4) had a 33% (4/12) and 75%

TABLE II. Chimerism according to stem cell source

		BM group		PBSC group	
Time after transplantation	**Level of chimerism**	**T-cell chimerism (%)**	**Myeloid chimerism (%)**	**T-cell chimerism (%)**	**Myeloid chimerism (%)**
1 mo	CC	80 (91)	82 (93)	47 (98)	47 (98)
BM (n = 88)	High-level MC	6 (7)	4 (5)	0	0
	Low-level MC	1 (1)	1 (1)	1 (2)	0
PBSC (n = 48)	Very low-level MC	1 (1)	1 (1)	0	1 (2)
6 mo	CC	46 (58)	48 (61)	35 (78)	37 (82)
BM (n = 79)	High-level MC	17 (22)	11 (14)	7 (16)	5 (11)
	Low-level MC	12 (15)	8 (10)	2 (4)	1 (2)
PBSC (n = 45)	Very low-level MC	4 (5)	12 (15)	1 (2)	2 (4)
1 y	CC	41 (72)	41 (57)	30 (73)	30 (73)
BM (n = 72)	High-level MC	17 (24)	6 (8)	9 (22)	6 (15)
	Low-level MC	10 (14)	11 (15)	0	2 (5)
PBSC (n = 41)	Very low-level MC	4 (5)	14 (19)	2 (5)	3 (7)
Last follow-up	CC	42 (64)	35 (53)	25 (71)	24 (69)
BM (n = 66)	High-level MC	16 (24)	7 (11)	7 (20)	7 (20)
	Low-level MC	4 (6)	7 (11)	1 (3)	1 (3)
PBSC (n = 35)	Very low-level MC	4 (6)	17 (26)	2 (6)	3 (8)

(3/4) incidence, respectively, of very low-level myeloid chimerism. T-cell chimerism was in the high chimerism ranges.

PBSC group. In contrast to the BM group, all the mMUDs (n = 17) have CC in all lineages. This is significant compared with the 33% incidence of very low-level myeloid MC in mismatched donors undergoing transplantation with BM as the stem cell source ($P = .03$).

GVHD

In patients undergoing transplantation with BM as the stem cell source, the incidence of aGVHD of grade II or greater was 25%. The incidence of grade III and IV aGVHD was low at 9%. Fifteen percent had cGVHD, of which 4% was classified as extensive.

As shown in Fig 3, the incidence of significant aGVHD (grade II or greater) was somewhat higher in the PBSC group at 31%, but this was not statistically significant compared with the BM group (31% vs 25%, $P = .42$). The overall incidence of grade III and IV aGVHD was also not significantly higher in the PBSC group (12% vs 9%, $P = .5$). Among the matched donors, only 5 (18%) of 28 had grade II or greater aGVHD, and only 1 (4%) of 28 patients had grade III or IV GVHD. However, patients who received mismatched donor PBSC transplants had a 48% (10/21) incidence of having GVHD of grade II or greater. This was significantly higher than the 18% (5/28) incidence of GVHD of grade II or greater in those receiving matched donor PBSC transplants ($P = .03$). The incidence of severe (grade III and IV) aGVHD was 24% (5/21) in mismatched donors. This was higher than the 4% (1/28) incidence of grade III and IV aGVHD in matched PBSC transplants, but this did not reach statistical significance, probably because of small numbers.

The incidence of cGVHD in the PBSC group was 24%, of which 16% was extensive; this was significantly higher in the PBSC group compared with the BM group ($P = .02$). This increased incidence of cGVHD was also seen exclusively in mismatched donors, where 10 (48%) of 21 had cGVHD, being extensive in 7 (33%) of 21. One patient had limited cGVHD, and 1 patient had extensive cGVHD in the matched PBSC group. All evaluable patients are off therapy for cGVHD with resolution of symptoms. One patient (PBSC group) has some joint restriction after resolution of sclerodermatous cGVHD.

Survival

Overall survival for the entire group at a median follow-up of 7.5 years was 78%.

BM group. As shown in Fig 4, *A*, 71 (76%) of 93 patients are alive at a median follow-up of 11.2 years. Causes of death in the 22 deceased patients were infection (n = 12), toxicity (n = 4), disease progression (n = 1), GVHD (n = 3), and others (n = 2).

PBSC group. Of 49 patients, 41 (84%) are alive at a median follow-up of 5.2 years. Causes of death in 8 deceased patients were infection (n = 2), toxicity (n = 2), and GVHD (n = 4).

There was no statistical difference in survival according to stem cell source (BM: 76% vs PBSC: 84%), nor was there any significant difference in survival according to donor type (Fig 4, *B*). The MUD, MMUD, MSD, MFD, and mMFD groups had survivals of 81%, 75%, 85%, 75%, and 71%, respectively.

Second procedures

Seven conditioned second transplantation procedures were performed for autologous reconstitution and return of disease at a median of 18 months after the first transplantation. Six patients received BM as a stem cell source for their first transplant. Four of 6 of these patients are alive and cured of their disease at last follow-up (1 had limited cGVHD). Two patients died of infectious complications during their second transplantation procedure.

Only 1 patient receiving PBSCs as a stem cell source required a second transplantation procedure. This patient with chronic granulomatous disease underwent an unsuccessful gene therapy procedure and then underwent a successful second Flu/Melph RIC transplantation procedure with 100% donor chimerism and is currently well and cured of his disease.

Five DLIs were performed (BM group, n = 2; PBSC group, n = 3). In 4 patients this resulted in stabilization/improvement of chimerism.

Three patients, all after BM transplantations, received CD34-selected boost transplants without conditioning to improve immune reconstitution. All these patients are alive, but 2 have ongoing suboptimal immune reconstitution.

Three patients underwent splenectomy (Wiskott-Aldrich syndrome with very low myeloid MC and thrombocytopenia, n = 1;

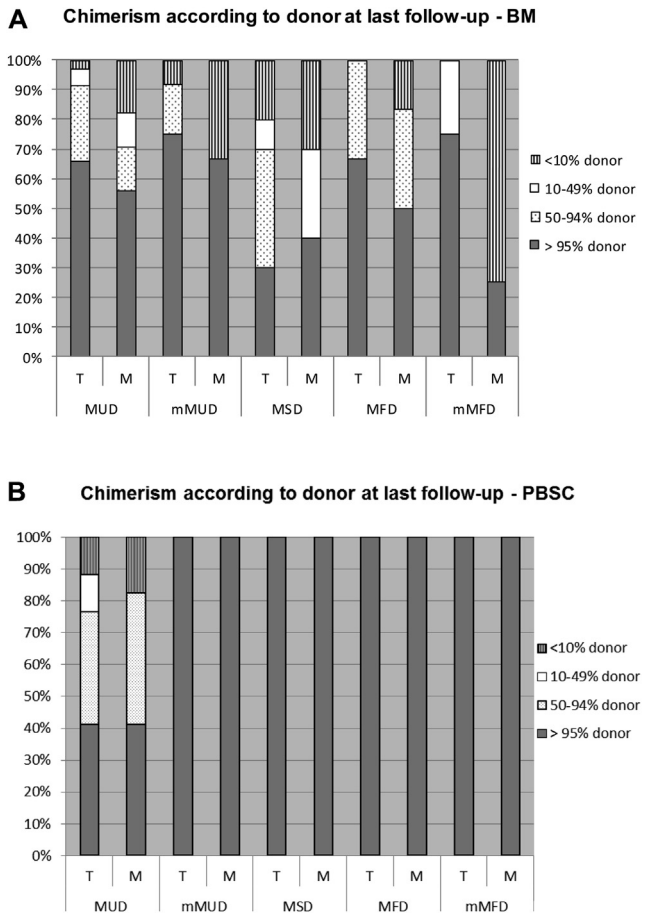

FIG 2. Chimerism according to donor source at last follow-up. **A,** With BM as the stem cell source, mMUDs had a 40% incidence of very low-level MC, which was most evident in the myeloid lineage. **B,** With PBSCs as the stem cell source, 100% of mismatched donors achieved complete donor chimerism in all lineages. MSDs also had a 30% incidence of very low-level MC in the myeloid lineage of the BM group. *M,* Myeloid engraftment; *T,* T-cell engraftment.

idiopathic thrombocytopenic purpura, n = 2). Platelet counts normalized after splenectomy in all 3 patients.

Outcomes in patients with very low-level MC within 1 year of HSCT

As shown in Table IV, 21 (15%) of 142 patients experienced very low-level MC at some point in the first year after transplantation. Eighteen (86%) of 21 of these patients had undergone transplantation with BM as the stem cell source. As shown in Fig 4, *C*, event-free survival in this group was significantly worse compared with the rest of the group (25% vs 70%, $P < .0001$). In addition to death, second procedures, splenectomy, cellular therapies, and return of disease manifestations were all considered events. Intravenous immunoglobulin replacement therapy alone was not considered an event. Seven (33%) of these 21 patients required a second transplantation procedure. Four were cured after the second procedure, 2 died, and 1 had an unsuccessful gene therapy procedure, followed by a second curative HSCT. Four (19%) of 21 patients have return of some disease manifestations and might need a second transplantation procedure in the future, and 2 (10%) of 21 had DLIs to improve chimerism (improved, n = 1; no improvement, n = 1). One patient with CD40 ligand deficiency died of progressive liver disease; 1 patient has partial disease correction and remains on intravenous immunoglobulin replacement; 1 patient with Wiskott-Aldrich syndrome underwent splenectomy, which normalized his platelet count, but continues to have very low-level MC and might be prone to autoimmune manifestations in the future; and 1 patient with hemophagocytic lymphohistiocytosis is clinically stable but with no evidence of donor engraftment so that his long-term prognosis remains guarded. Three of 21 patients (all with SCID) are well and off immunoglobulin replacement, and 1 patient was lost to follow-up.

On multivariate analysis, perhaps because of the small sample size, none of the predictors analyzed for very low-level MC (age at transplantation, diagnosis, source of stem cells, type of donor, or year of transplantation) were significant variables.

DISCUSSION

The level of engraftment that is curative after HSCT depends on the disease type and lineages affected. In patients with diseases like SCID, T-cell engraftment is crucial, whereas in those with other PIDs, such as chronic granulomatous disease and leucocyte adhesion deficiency, myeloid engraftment is important for disease cure. In patients with PIDs, it has been shown that long-term well-being and durable immune reconstitution require adequate levels of true stem cell engraftment, as evidenced by continuing donor myeloid chimerism.[7,8] Hence an ideal RIC HSCT regimen should not only ensure low levels of procedure-related toxicity but also secure sustained levels of stem cell engraftment.

In our study patients undergoing transplantation with BM as a stem cell source had a higher incidence of very low-level MC in the myeloid lineage. These patients had much worse event-free survival compared with the rest of the group, with only 3 of 21 patients being free of disease after their primary transplantation procedure. In addition, in the long term, they are at an increased risk of graft exhaustion and return of disease manifestations. In contrast, long-term donor chimerism was improved in the PBSC group, with only 7% having very low-level MC.

PBSC grafts typically contain 1 log more $CD34^+$ stem cells and 1 log more T cells than BM. Therefore the higher levels of donor engraftment observed with PBSCs are likely to reflect a combination of both an increased alloreactive "graft versus marrow" effect mediated by T cells and greater donor stem cell competition for niches in the BM. The relative contribution of these 2 factors is not known, but together, they appear to reduce the risk of autologous reconstitution. In one of the few studies comparing stem cell sources in the nonmyeloablative setting, Dey et al[9] compared PBSCs to BM as a stem cell source in 54 adults with hematologic malignancies. Consistent with our findings, they also observed higher levels of donor chimerism in the PBSC group (83% vs 38%). Similarly, rates of graft loss were also significantly lower in the PBSC group (8% vs 37%).

Lineage-specific chimerism analysis of our group led us to identify 2 problem groups of patients: those undergoing transplantation with mMUDs with BM as a stem cell source and those undergoing transplantation with MSDs (all but 1 sibling transplantation was done with BM as the stem cell source). Although survival in these groups was comparable with that in the rest of the patients, the incidence of very low levels of MC was significantly higher in both these cohorts.

TABLE III. Chimerism according to donor source at last follow-up

		BM group		PBSC group	
Donor	**Level of chimerism**	**T-cell chimerism (%)**	**Myeloid chimerism (%)**	**T-cell chimerism (%)**	**Myeloid chimerism (%)**
MUD	CC	23 (66)	19 (54)	7 (41)	7 (41)
	High-level MC	9 (26)	5 (14)	6 (35)	7 (41)
BM (n = 35)	Low-level MC	2 (6)	4 (12)	2 (12)	0
PBSC (n = 17)	Very low-level MC	1 (3)	6 (17)	2 (12)	3 (18)
mMUD	CC	9 (75)	8 (66)	17 (100)	17 (100)
	High-level MC	2 (17)	0	0	0
BM (n = 12)	Low-level MC	0	0	0	0
PBSC (n = 17)	Very low-level MC	1 (8)	4 (33)*	0	0
MSD	CC	3 (30)	4 (40)	0	0
	High-level MC	4 (40)	0	1 (100)	1 (100)
BM (n = 10)	Low-level MC	1 (10)	3 (30)	0	0
PBSC (n = 1)	Very low-level MC	2 (20)	3 (30)	0	0
MFD	CC	4 (67)	3 (50)	2 (100)	2 (100)
	High-level MC	2 (33)	2 (33)	0	0
BM (n = 6)	Low-level MC	0	0	0	0
PBSC (n = 2)	Very low-level MC	0	1 (17)	0	0
mMFD	CC	3 (75)	1 (25)	1 (100)	1 (100)
	High-level MC	0	0		
BM (n = 4)	Low-level MC	1 (25)	0		
PBSC (n = 1)	Very low-level MC	0	3 (75)		

*Statistically significant compared with mMUDs in the PBSC group: $P = .03$.

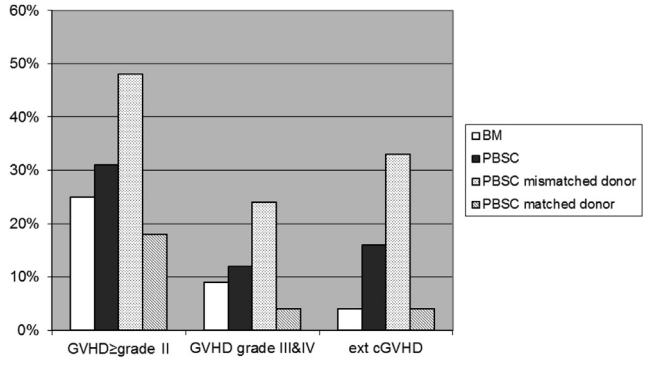

FIG 3. GVHD after BM and PBSC transplantations. Incidence of significant (grade II or greater), severe aGVHD (grade III and IV), and cGVHD was low with BM transplants. There was a significantly higher incidence of aGVHD and cGVHD with PBSC transplants from mismatched donors. The incidence of GVHD with PBSC transplants from matched donors was low and similar to that in the BM group. The incidence of severe GVHD was only 4% in the matched PBSC setting.

The number of patients undergoing transplantation with an MSD in our study was small (14 patients; 13 had BM as a stem cell source); however, we observed that 30% of these patients had very low-level myeloid engraftment, and 1 additional patient died of return of hemophagocytic lymphohistiocytosis. All patients with very low-level MC have had to undergo second transplantation procedures. The relatively small stem cell dose acquired from pediatric sibling donors together with insufficient T-cell alloreactivity in this predominantly chemo-naive group of patients might have contributed to this increased incidence of graft loss and poor myeloid engraftment in the RIC setting in children. Although there are data on the safety and efficacy of obtaining PBSCs from pediatric sibling donors,[10] this is not routine practice in the United Kingdom and some other countries. One option for improving engraftment in this group of patients might be to omit/reduce the dose of alemtuzumab or administer it earlier in conditioning, thereby causing less T-cell depletion of the graft and enabling greater graft-versus-marrow alloreactivity. Alternatively, other RIC protocols might be preferable for patients with PIDs receiving transplants from MSDs, such as recently reported by Gungor et al,[11] who observed excellent outcomes and high levels of engraftment using a combination of submyeloablative doses of busulfan and fludarabine in a cohort of 56 patients undergoing transplantation for chronic granulomatous disease. This cohort included 21 MSD transplantations, and their outcome and engraftment results were comparable with those of the rest of the group.

Patients receiving transplants from mismatched donors with BM as the stem cell source also had a high incidence of very low-level myeloid MC (33%). This is consistent with data from adult studies in which graft rejection has been a significant problem in the RIC setting using mismatched donors.[12-14] The effect of the mismatch can be overcome by increasing the CD34 dose and the alloreactivity of the graft; both these goals are met by using PBSCs, and the majority of adult RIC protocols now use PBSCs as the preferred stem cell source. In children there has been a gradual but similar shift in practice, but there is a paucity of published literature on stem cell sources in the RIC setting in pediatrics.

Between 1998 and 2002, BM was the predominant stem cell source used for HSCT in our cohort of mismatched donors. In view of the high incidence of rejection and very low-level MC in the mismatched donor group, from 2002 onward, on the basis of adult experience, we made 2 changes to our approach in transplantation with mismatched donors. First, we switched to using PBSCs as our preferred stem cell source, and on the basis of the experience of the Seattle group, we changed our GVHD prophylaxis to include MMF as a proengraftment agent.[15] After this change in practice, we have had no rejections in the mMUD group, and 100% of patients achieved complete donor chimerism in all lineages.

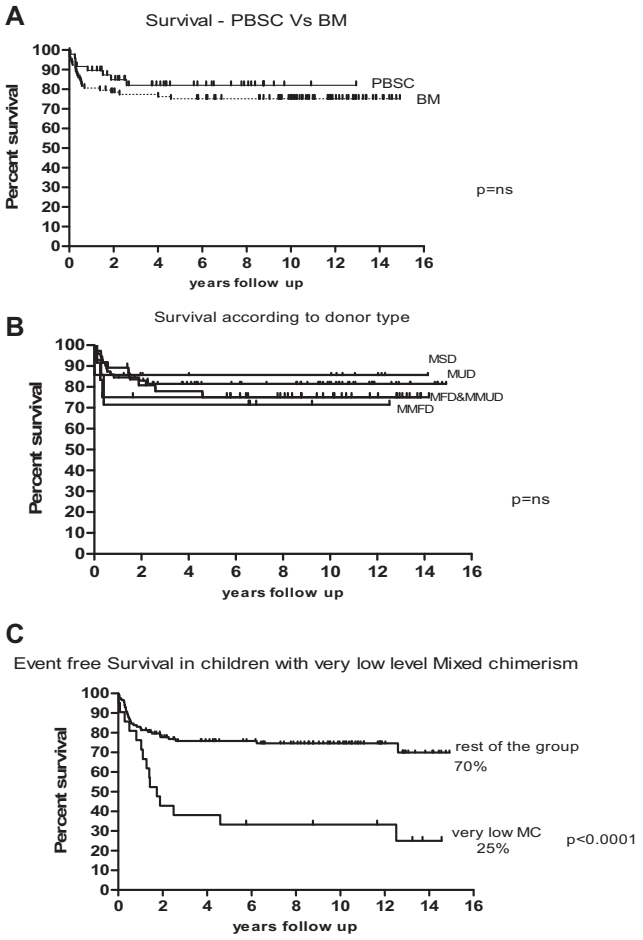

FIG 4. Overall survival in the BM and PBSC groups, event-free survival in patients with less than 10% donor chimerism compared with patients with greater than 10% donor chimerism, and survival according to donor type are shown. Survival was very good in the BM and PBSC groups at 76% and 84%, respectively. There was no statistical difference in survival according to donor type. Patients with less than 10% donor chimerism had significantly poorer event-free survival at only 25% compared with 70% in patients with higher levels of chimerism.

However, this improvement came at the cost of excessive aGVHD and cGVHD, which is of no beneficial value to this patient group. In our study this high incidence of severe aGVHD and cGVHD was restricted to PBSC transplants from mMUDs. With matched donors, the incidence of GVHD was low and equivalent in the BM and PBSC groups. The persistence of host antigen-presenting cells after RIC might contribute to the pathogenesis of GVHD,[16,17] and this is likely to be compounded in the presence of an antigenic mismatch. Our findings are consistent with those of other groups reporting high rates of cGVHD with mismatched donors in the RIC setting.[18,19]

Although there are multiple factors contributing to the pathogenesis of GVHD after PBSC transplantation,[20] one option to reduce GVHD might be to limit the number of T cells in the PBSC graft. This could be achieved by enriching the stem cell collection by means of $CD34^+$ cell selection and adding back the $CD34^-$ cell population to contain a fixed T-cell dose. Currently, we are studying this approach in our unit, and preliminary results are encouraging.

Another option for reducing GVHD in this patient group might be to increase the dose of alemtuzumab. A study by Mead et al[21] in adults with hematologic malignancies using an identical RIC protocol but with a total dose of 100 mg of alemtuzumab (approximately twice the amount in our study) found no difference in the incidence of GVHD between HLA-matched and mismatched donors. However, the slow immune reconstitution after this dose of alemtuzumab might be problematic in our cohort of patients, many with ongoing viral infections at the time of HSCT. A further option for this group of patients might be to use granulocyte colony-stimulating factor–primed BM allografts. This approach might combine the benefits of PBSC transplantation (low rejection and fast cell recovery) with those of BM transplantation (low incidence of cGVHD). Morton et al,[22] in their prospective randomized study comparing granulocyte colony-stimulating factor–primed BM allografts with PBSC transplants in matched donors, report comparable engraftment in both arms but with a significant reduction in the incidence of cGVHD in the granulocyte colony-stimulating factor–primed BM arm. The study was closed after the interim analysis at 6 months because the study's end point of significant cGVHD had been reached. Larger studies with longer follow-up evaluating the benefit of this approach and documenting donor safety are necessary before it can be recommended for routine use. It is possible that other reduced-toxicity protocols, such as that reported by Gungor et al,[11] might provide adequate engraftment with acceptable GVHD rates in mismatched donors.

In summary, our RIC regimen of fludarabine and melphalan resulted in durable engraftment in the majority of patients and comparable overall survival in the BM and PBSC groups. However, when BM was used as the stem cell source, higher rates of very low-level MC, particularly in the myeloid lineage, were observed than with PBSCs, and this was associated with poor event-free survival. Patients with matched donors had a low incidence of GVHD and achieved excellent long-term engraftment in all lineages by using PBSCs, and this would be our preferred stem cell source for matched donors. Patients with mismatched donors remain a difficult group of patients for transplantation, experiencing poor engraftment (with BM) and high levels of GVHD (with PBSC), and for this group, we have proposed some potential strategies. Patients receiving MSD transplants also do not achieve good levels of engraftment with our Flu/Melph RIC regimen, and we are currently trialing alternative RIC protocols for this group.

Our study has the limitations of a heterogeneous patient population and small sample size, and hence we could not conclusively demonstrate a relationship between chimerism and stem cell source in multivariate analysis. It could be argued that the better chimerism results seen in the PBSC group were partly due to the introduction of molecular methods of tissue typing from 2002 onward; however, the fact that the incidence of autologous reconstitution and very low-level MC did not change in the 2 time periods suggests that this was possibly not a major confounding factor. Larger prospective studies are needed to further validate our findings, to study the effect of Flu/Melph pharmacokinetics on chimerism, and to study the disease-specific implications of MC.

TABLE IV. Characteristics of patients with very low-level MC within 1 year of transplantation

UPN	Diagnosis	Age at transplantation (y)	Donor	Stem cell source	Outcome
GOS006	SCID	0.77	MUD	BM	Well, off immunoglobulin replacement
GOS014	T-cell immunodeficiency	0.77	mMUD	BM	Well, off immunoglobulin replacement
GOS016	CID	0.32	MUD	BM	Successful second transplantation
GOS018	SCID	0.55	mMUD	BM	Chronic lung disease; remains on immunoglobulin replacement
GOS020	SCID	1.46	mMUD	BM	Immunoglobulin replacement therapy
GOS028	SCID	0.46	mMFD	BM	Chronic lung disease; remains on immunoglobulin replacement
GOS029	CID	10.6	MSD	BM/PBSC*	Ongoing disease manifestations, severe warts, lymphoedema
GOS033	CD40 ligand deficiency	15.9	mMUD	BM	Died of progressive liver disease
GOS037	SCID	6.3	mMUD	BM	Died after second transplantation
GOS044	SCID	0.9	MUD	BM	Well, off immunoglobulin replacement
GOS048	SCID	0.3	MUD	BM	Successful second transplantation
GOS068	CD40 ligand deficiency	1.3	MSD	BM	Successful second transplantation
GOS076	WAS	2.1	MUD	BM	Splenectomy with normalization of platelet count
GOS087	SCID	1.3	MUD	BM	Lost to follow-up
GOS099	Phagocytic disorder	5.2	MFD	BM	Ongoing skin infections
GOS088	Phagocytic disorder	4.3	MUD	PBSC	Failed gene therapy; successful second transplantation
GOS108	HLH	1.3	MUD	PBSC	DLI with stabilization of chimerism
GOS111	HLH	0.76	MUD	BM	DLI with improvement in chimerism
GOS005	XLP	3.6	MSD	BM	Died after second transplantation
GOS031	WAS	2.5	MSD	BM	Successful second transplantation
GOS113	HLH	1.6	mMUD	BM	Well

CID, Combined immunodeficiency; *HLH*, hemophagocytic lymphohistiocytosis; *WAS*, Wiskott-Aldrich syndrome; *XLP*, X-linked lymphoproliferative disorder.
*This patient received BM, which was then topped up with PBSCs because of low BM stem cell numbers. For analytic purposes, he is included in the PBSC group.

Key messages

- **Long-term myeloid chimerism can be inadequate in a significant number of children undergoing transplantation for PIDs using RIC with Flu/Melph when BM is used as the stem cell source.**
- **This results in inferior event-free survival.**
- **Using peripheral blood as the stem cell source in fully matched donors can abrogate this problem associated with the Flu/Melph conditioning regimen.**

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