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Neutrophil function in children following allogeneic hematopoietic stem cell transplant

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Abstract

HSCT is a lifesaving procedure for children with malignant and non-malignant conditions. The conditioning regimen renders the patient severely immunocompromised and recovery starts with neutrophil (PMN) engraftment. We hypothesize that children demonstrate minimal PMN dysfunction at engraftment and beyond, which is influenced by the stem cell source and the conditioning regimen. Peripheral blood was serially collected from children at 1 to 12 months following allogeneic HSCT. PMN superoxide (O_2^-) production, degranulation (elastase), CD11b surface expression, and phagocytosis were assessed. Twenty-five patients, mean age of 10.5 yr with 65% males, comprised the study and transplant types included: 14 unrelated cord blood stem cells (cords), seven matched related bone marrow donors, three matched unrelated bone marrow donors, and one peripheral blood progenitor cells. Engraftment occurred at 24 days. There were no significant differences between controls and patients in PMN O_2^- production, phagocytosis, CD11b surface expression, and total PMN elastase. Elastase release was significantly decreased <6 months vs. controls ($p < 0.05$) and showed normalization by six months for cords only. The conditioning regimen did not affect PMN function. PMN function returns with engraftment, save elastase release, which occurs later related to the graft source utilized, and its clinical significance is unknown.

Keywords

neutrophil dysfunction; elastase release; hematopoietic stem cell transplantation

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Conflict of interest

None of the authors have a financial or other conflict of interest with the presented data or findings of this manuscript. Dr. Silliman has received both honoraria and research funding from Pall Corporation and New Health Sciences, Inc., which are not related to any of the reported data in this manuscript.

Authors' contributions

MWK accrued all patients and control subjects, performed all of the experiments, drafted the figures, tables, and the manuscript. MRK helped to complete all of the experiments and helped to draft the figures, tables, and the manuscript. CCS provided technical expertise for all experiments, was instrumental in the concept and design of the study, and guided all experimentation, data analysis, and drafting of the manuscript. RQ was paramount to the concept/design of the project, helped to accrue all patients, was key to all data collection and analysis, and helped to complete the manuscript with important critical revision.

HSCT is a lifesaving therapy for a variety of malignant and non-malignant conditions. The first hallmark of a successful HSCT is engraftment, which is generally considered to be a sustained ANC > 500 (1–3). Both engraftment and prevention of GVHD require a preparative conditioning regimen, which leads to a period of significant immune suppression (4).

Opportunistic infections lead to increased morbidity and mortality in BMT recipients (5). Crucial to avoiding infections is recovery of a functional immune system with T and B lymphocyte immune competence, comprised of both cell-mediated and humoral function (6). Neutrophils (PMNs) are the first cells to return, are the harbinger of engraftment, and represent the initial return of potential, *in vivo* protection (7). Once a stable ANC of 500 is reached, antibiotic prophylaxis is often discontinued; however, cessation of antimicrobial prophylaxis may not necessarily be wise, as functionality of these newly engrafted PMNs is unknown (8).

Multiple studies of adult HSCT patients have demonstrated that these early PMNs are not fully functional and may remain dysfunctional for >6 months (8–13). Although this PMN dysfunction in adults has been well characterized, it has not been fully evaluated in children (8). Furthermore, potential differences between HSCT in children vs. adults are likely related to differences in the intensity of conditioning regimens, which are more frequently myeloablative in pediatric patients. The only post-HSCT study of PMN function on children alone focused on two azurophilic granule proteins: (i) BPI and (ii) defensins (14). Therefore, we hypothesize that children demonstrate minimal PMN dysfunction at the time of engraftment and beyond, which is influenced by the stem cell source and the conditioning regimen.

Materials and methods

Materials

All chemicals unless otherwise specified were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). Phagotest™ was purchased from Glycotope (Berlin, Germany). All antibodies for flow cytometry were purchased from BD Biosciences (San Jose, CA, USA). The Diogenes®- Cellular Luminescence Enhancement System for Superoxide Detection was purchased from National Diagnostics (Atlanta, GA, USA). An FCS Canto II five-color flow cytometer was purchased from Beckman Dickinson (San Jose, CA, USA).

Study population

Patients, 1–21 yr, were recruited from children requiring allogeneic HSCT at the Center for Cancer and Blood Disorders, Children's Hospital Colorado, Aurora, CO. Informed consent was obtained from these patients, healthy sibling controls, healthy adult controls, and their families either prior to or shortly after HSCT employing a protocol approved by the Colorado Multiple Institution Review Board (COMIRB) in either the inpatient or outpatient setting. For all patients eligible to provide informed assent, an assent was obtained, which was a necessary part of the aforementioned COMIRB protocol. All patients enrolled had to undergo an allogeneic HSCT and were eligible irrespective of the underlying diagnoses.

Exclusion criteria

Patients aged 1–21 yr were eligible for enrollment, and the amount of blood drawn was limited by the patient weight. The controls were made up of healthy siblings (6) and healthy adults (11). HSCT patients with ANC < 500 had sampling deferred from this time point due to low cell yields such that sufficient PMNs could not be collected to assess all PMN functions. Most patients with an ANC < 500 were at the one-month time point. Lastly, patients receiving systemic steroids ≥ 2 mg/kg/day, or in ICU at time of collection had sample collection deferred due to their clinical condition or to the possible effects of steroids on PMN function (15–20).

Whole blood collection

About 2–10 mL of peripheral blood was obtained in plastic sodium heparin tubes, at 1, 2, 3, 6, and 12 months post-HSCT at Children's Hospital Colorado. A number of patients were present on the same day, and in some cases, two patients were drawn. For all subjects, at least one healthy control was drawn at the same time.

Neutrophil isolation

On the day of collection, PMNs were isolated from heparinized whole blood by Ficoll-Hypaque differential centrifugation and hypotonic lysis (21). PMNs were counted using an AcT cell counter (Beckman Coulter, Brea, CA, USA) and resuspended in KRPD buffer (Krebs–Ringers phosphate with 2% dextrose, pH 7.35) at a concentration of 2.5×10^7 cells/mL (21).

CD11b surface expression

PMNs were stimulated with buffer or 2 μ M PAF for five min at 37 °C and then incubated with a PE-labeled antibody to CD11b or PE-labeled isotype controls for 30 min at 4 °C (22). CD11b surface expression was measured by flow cytometry, and the data are expressed as the mean fluorescence intensity as previously reported (22).

Superoxide anion production

The maximal rate of superoxide anion (O_2^-) production (nmol/min) was measured using Diogene®-based chemiluminescence (23). Isolated PMNs were primed with buffer (control) or 2 μ M PAF for three min at 37 °C and then activated with 1 μ M fMLF for three min. The data are expressed as fold increase over buffer/fMLF for the PAF-primed fMLF activation of the NADPH oxidase (23).

Degranulation assays

Isolated PMNs were primed with buffer or 2 μ M PAF for three min at 37 °C and then activated with buffer or fMLF (22). The PMNs were removed by centrifugation, and elastase was measured employing a specific colorimetric assay over 60 min at 37 °C, as previously published (22). Total cellular elastase was measured by dissolving cellular membranes with 1% Triton X-100 at 37 °C for 30 min, and the total cellular elastase in the supernatant was measured (22).

Phagocytosis

Phagocytosis analysis was performed on whole blood samples via the commercially available Phagotest™ flow cytometry-based assay per the manufacturer's instructions (24). This test employs intact, non-pathogenic FITC-labeled *E. coli*. The percentage of the total number of intracellular ingested *E. coli* was calculated per the known number of fluorescently labeled bacteria added and directly compared to the paired, control PMNs.

Statistics

The data are presented as the mean $\pm$ the standard error of the mean (s.e.m.). Statistical differences were determined by employing a paired or independent analysis of variance with a Bonferroni or Newman-Keuls *post hoc* test for multiple comparisons, depending upon the equality of variance. Statistical significance was determined at the $p < 0.05$ level except for O_2^- production in which the PAF/fMLF response was normalized to the buffer/fMLF response, such that the significance was lowered to $p < 0.01$.

Results

Study population

Twenty-seven patients were enrolled in the study (Table 1). Two patients died before a sample was collected and were excluded. Seven patients died of varying causes including infection, relapse, and GVHD (grade IV, patient #13) during the collection period, resulting in an overall survival of 72%, similar to the one-yr survival rate of 75% for the parent center during the study period. The mean age of participants enrolled was 10.5 yr old, with 65% being male. The source of allogeneic hematopoietic stem cells was unrelated cord blood (cords) for 14 patients, bone marrow for 10, of which two were unrelated donors, and one patient received peripheral blood stem cells from an unrelated donor. The indication for transplant was primarily malignancy vs. non-malignancy (18 vs. 7). Malignant diagnoses included high-risk AML (in CR1; $n = 3$; in CR2 or 3; $n = 8$; relapse; $n = 1$), secondary AML (in CR1; $n = 1$), high-risk ALL (in CR1; $n = 1$; in CR2; $n = 2$), non-Hodgkin's lymphoma (in CR2; $n = 1$), and monosomy 7 in a patient with Noonan's syndrome who had recovered from a low-grade astrocytoma ($n = 1$). Non-malignant diagnoses included common variable immunodeficiency with concomitant severe aplastic anemia ($n = 1$), epidermolysis bullosa ($n = 1$), Wiskott-Aldrich syndrome ($n = 1$), osteopetrosis ($n = 1$), aplastic anemia ($n = 1$), sickle-cell disease ($n = 1$), and cartilage-hair hypoplasia with severe immune deficiency ($n = 1$). The GVHD prophylaxis regimens were very similar with 13 cord blood recipients on cyclosporine + MMF with one on cyclosporine alone while nine of the bone marrow recipients received cyclosporine + MMF, one had tacro + MMF, and the last cyclosporine alone. All patients received corticosteroids briefly, and there were no statistical differences in the GVHD prophylaxis regimens between the groups (cord vs. bone marrow recipients) at the time blood was obtained.

The initial plan per protocol was to recruit healthy siblings as the control group; however, due to multiple reasons: not being native to the Denver area, lack of siblings, sibling refusal, this was later deemed not feasible. Therefore, healthy adult controls (<50 yr of age) were recruited as an alternative. In a small number of willing sibling participants ($n = 7$), serial

samples were obtained. The number of controls was 17 (7–49) yr of age with a median age of 34 yr. Controls were run on the same days as the patient samples, and multiple patient samples were often run concomitantly, when scheduling permitted. In regard to all the assays performed in this study, there was no difference noted among gender or age, as expected (results not shown); because after 12 months of age, innate immunity with respect to PMN function is mature and is not altered until the geriatric age group (>70 yr) (25–27).

The number of patients collected at each time point decreased over time as patients passed away, missed appointments, or relocated to their primary oncology program (Table 2). The mean ANC increased with time, with only one patient requiring G-CSF outside the one-month time point and the overall rate of GVHD, grade II, was <25%. The incidence of bacterial and fungal infections decreased with time post-HSCT, likely the result of immune system recovery and decreased immune suppression (Tables 1 and 2).

Surface expression of CD11b

The baseline surface expression of CD11b was not significantly different vs. controls at any of the time points. PAF-induced increases in CD11b surface expression were also not significantly different from control levels at any time point post-HSCT (Fig. 1).

O_2^- production

Maximal rate of O_2^- production in PAF-primed PMNs activated with fMLF from HSCT patients was not significantly different from identically treated PMNs from healthy controls at any time point (Fig. 2). There was a trend toward increased O_2^- production with time post-HSCT in the study group, which was not significant, vs. the controls (Fig. 2).

Degranulation

As compared to the buffer-treated controls, activation with fMLF produced significantly increased amounts of elastase released (Fig. 3a). Furthermore, PAF priming followed by fMLF activation of PMNs further increased elastase release vs. both the buffer-treated controls and fMLF-activated PMNs ($p < 0.05$) (Fig. 3a). In contrast, significantly less elastase was released from primed and primed/activated PMNs samples in the patients following transplantation at one, two, and three months post-HSCT vs. the healthy controls. When all the HSCT patients are included in the analyses, the PMN elastase release was not statistically different from the controls at six months and one yr post-HSCT (Fig. 3a). The total cellular elastase within the PMNs was also measured in both patient and control groups, and there was no significant difference between the controls and patients post-HSCT, indicating that the observed defect was confined to elastase release and not production (Fig. 3b). In addition, when the PMNs from children who received cord blood stem cells were compared to the paired PMNs from healthy controls, the defect in elastase release was only statistically different at one and three months and was not statistically decreased from controls at later time points. In contrast, the significant decrease in elastase release was present throughout the study period in the PMNs from children that had received bone marrow or peripheral blood stem cells (Fig. 4). Lastly, TBI- vs. busulfan-based regimens were evaluated to investigate any contribution to this decrease in elastase release; however, no differences were found (results not shown).

Phagocytosis

PMN phagocytosis of non-pathogenic, FITC-labeled *E. coli* demonstrated that there were no significant differences in bacterial ingestion between the HSCT patient samples and controls (Fig. 5).

Discussion

In children following allogeneic HSCT, PMN function was similar to the PMNs from healthy controls for most aspects, including the respiratory burst, phagocytosis, and CD11b surface expression both at rest or following PAF priming. There was a defect in elastase release post-HSCT, which was the most pronounced following allogeneic HSCT with bone marrow. Importantly, the total cellular elastase in the PMNs was not different between the HSCT patients and healthy controls indicative of elastase release rather than production. In this series, the number of bacterial and fungal infections also decreased with time, likely due to the return of PMN number and function and a decrease in immune suppression. A few samples were missed due to the exclusionary criteria, especially infections or an ANC < 500.

In children, plasma levels of PMN elastase are an early indicator of septicemia and bacterial meningitis, and elastase is known to be involved in the formation of NETs to effectively engulf and kill pathogenic bacteria (28, 29). In the absence of elastase, NET formation is inhibited, predisposing the affected individual to bacterial infections (28). Thus, the precise role of elastase in defense against bacterial and fungal infections has not been defined except in light of a congenital deficiency and NET formation (30). Human PMN elastase is thought to have both pro- and anti-inflammatory effects (31). The pro-inflammatory effects include increased chemotaxis with decreases in the degradation of E and VE cadherin, leading to pulmonary edema, and the anti-inflammatory effects include the degradation of pro-inflammatory cytokines, for example, IL-1 β , TNF- α , and IL-2 (31). Furthermore, mutations in the ELANE gene, which makes elastase, as well as genes making proteins that interact with PMN elastase, are clinically associated with severe congenital neutropenia and cyclic neutropenia (30, 32, 33). Elastase also has intracellular transporter function and the mutated elastase becomes “inappropriately located” and dysfunctional, which may have little to do with children post-HSCT (30, 32, 33). Children with the Chediak–Higashi syndrome have a degranulation defect, including elastase release, and low levels of PMN cathepsin G, both of which contribute to their inherent immune deficiency (34). Elastase release defects are not confined to patients with inherited disorders, as such defects have been observed in *ex vivo* differentiated PMNs from hematopoietic stem cells (35).

Because of the relatively low incidence of GVHD, both acute and chronic, in this case series, one may question whether decreased elastase conferred a resistance to GVHD. The number of patients is small, and the relative lack of significant GVHD precluded definitive assessment as to the role of PMN elastase. Moreover, these data reflect a large number of patients who received cord blood grafts which may be the reason that relatively few patients had GVHD.

There was no difference in the baseline or PAF-induced surface expression of CD11b, an integrin subunit that pairs with CD18 to form the β_2 -integrin CD11b/CD18, which is present in the gelatinase-rich granules between the children post-HSCT and the controls (36–40). Although required for PMN emigration and chemotaxis, CD11b/CD18 surface expression is not a distinct measure of chemotaxis (36–40).

G-CSF-mobilized PMNs from healthy adults displayed alterations in PMN functions (41). The PMNs exhibited a modest chemotactic defect, a decreased respiratory burst in PAF-primed PMNs activated with fMLF, and reduced killing of *S. aureus* at a 10:1 ratio (bacteria:PMN) (41). As all patients in this study were receiving G-CSF until the ANC > 500, PMN function at the one-month time point may exhibit the noted alterations in PMN due to G-CSF; however, there was no decrease in O_2^- production to PAF/fMLF observed, and all patients, save one, were drawn >1 wk after G-CSF was discontinued. In addition, BPI and defensins, which are azurophilic granule proteins, have been reported to be decreased in children post-HSCT (14, 41). However, in the reported data, the elastase content in the azurophilic granules was identical between children post-HSCT and controls, dissimilar to these previous data (14, 41).

There are a number of limitations in this study, including the preponderance of unrelated cord blood transplants. Our center has a preference for unrelated cord blood as an HSCT source; however, even if the differences between cord blood and bone marrow transplants were ignored, a difference in elastase release was still seen in the aggregate. The reported data are from a single center with small numbers, which made analysis of several aspects difficult, particularly the role of (i) GVHD, (ii) the conditioning regimen, and (iii) the elastase release defect with relation to the stem cell source. A multi-institutional study should confirm these results, especially the defect in elastase release. In addition, the allowable volume of blood was not sufficient to exhaustively examine PMN function, especially chemotaxis or bacterial killing. To date, there is little emphasis on the qualitative role of the PMN, with significant emphasis on PMN numbers. Clinical studies of oncology patients treated with G-CSF have demonstrated a decrease in infections with improved morbidity and mortality, when the absolute neutrophil count > 500 (2–4, 41, 42). The PMNs from children post-HSCT were remarkably intact such that the emphasis on PMN number may be clinically relevant.

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Abbreviations

ALL	acute lymphoblastic leukemia
AML	acute myelogenous leukemia
ANC	absolute neutrophil count
BPI	bactericidal permeability increasing protein

CR1	first clinical remission
CR2	second clinical remission
fMLF	formyl-methionyl-leucyl-phenylalanine
G-CSF	granulocyte colony-stimulating factor
GVHD	graft-vs.-host disease
HSCT	hematopoietic stem cell transplant
MMF	mycophenolate mofetil
NETs	neutrophil extracellular traps
PAF	platelet-activating factor
tacro	tacrolimus
TBI	total body irradiation

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Fig. 1.

PMN CD11b: Baseline and stimulated surface expression. The mean fluorescence intensity (MFI) of 10 000 events is depicted as a function of treatment group as defined as the PMNs from healthy controls and children who underwent allogeneic HSCT and drawn one, two, three, six, and 12 months following transplantation. No significant differences were present between the controls and any time point post-bone marrow transplantation for the baseline and stimulated CD11b surface expression ($p > 0.05$, controls $n = 13$, one month $n = 21$, two months $n = 13$, three months $n = 12$, six months $n = 8$, 12 months $n = 8$). In addition, there was a statistical increase in CD11b surface expression in the PAF-stimulated PMNs vs. the buffer-treated controls for each pairing; for example, the controls and all time points one, two, three, six, and 12 month samples post-HSCT; in some cases, one control served for more than one transplanted child (* $p < 0.05$ vs. buffer stimulated PMNs).

Fig. 2.

PMN superoxide anion production. The PAF-primed, fMLF-activated respiratory burst, measured as the fold increase over fMLF of the maximal rate of O_2^- production, is depicted as a function of treatment group. There were no significant differences in PAF/fMLF-induced O_2^- production between the controls and all time points post-allogeneic bone marrow transplantation ($p > 0.05$, control $n = 17$, one month $n = 21$, two months $n = 19$, three months $n = 15$, six months $n = 11$, 12 months $n = 8$; in some cases, one control served for more than one transplanted child).

Fig. 3.

PMN elastase release. (a) The percentage of elastase released is shown as a function of treatment group. Firstly with regard to the controls, the PMNs activated with 1 μ M fMLF demonstrated an increase amount of elastase release vs. buffer-treated controls, and 2 μ M PAF priming and fMLF activation elicited a further increase in elastase release that was significantly greater than either buffer or fMLF-activated PMNs ([†] $p < 0.05$ vs. buffer controls and [‡] $p < 0.05$ vs. fMLF). In the buffer-treated PMNs, there is no difference between the controls (black bars) and any time point post-allogeneic HSCT. However, PMNs activated with fMLF or primed with (PAF) and activated with 1 μ M fMLF demonstrated a decreased amount of elastase released at 1–3 months post-HSCT vs. buffer-treated controls ($*p < 0.05$). This difference disappears at six and 12 months post-HSCT ($p > 0.05$, controls $n = 17$, one month $n = 21$, two months $n = 17$, three months $n = 15$, six months $n = 11$, 12 months $n = 8$). (b) Total cellular elastase is depicted as a function of treatment group. The total PMN elastase from Triton-X-treated control PMNs (black bar) was not different from the total PMN elastase from the PMNs from children post-HSCT (gray bar), ($p > 0.05$, controls $n = 17$, children post-HSCT $n = 25$; in some cases, one control served for more than one transplanted child).

Fig. 4.

PMN elastase release: Children transplanted with cord blood stem cells vs. children transplanted with bone marrow and peripheral stem cells. Total PMN elastase release is shown as a function of treatment group. The controls are represented by a black bar, the children transplanted with bone marrow and stem cell grafts by dark gray bars, and children transplanted with cords by light gray bars. The PMNs from children transplanted with bone marrow or peripheral blood stem cells had significantly less elastase released compared to controls at every time point through 12 months post-HSCT. The PMNs from children transplanted with cord blood stem cells showed normalization of elastase release to control levels by six months post-HSCT (* $p < 0.05$ vs. controls, bone marrow/peripheral stem cell grafts $n = 9$, cord blood grafts $n = 15$).

Fig. 5. Phagocytosis by PMNs from children post-allogeneic HSCT. The percentage of the total labeled *E. coli*, which was phagocytized (intracellular), is depicted as a function of treatment group. Phagocytosis did not significantly differ from controls (black bar) at any time point ($p > 0.05$, controls $n = 17$, one month $n = 20$, two months $n = 16$, three months $n = 15$, six months $n = 11$, and 12 months $n = 8$; in some cases, one control served for more than one transplanted child).

Table 1

Patient demographics, diagnosis, and treatment

	Sex	Age	Diagnosis	Preparative regimen	Donor type	GVHD	Stem cell dose* TNC/kg (10 ⁷)/ CD34/kg (10 ⁵)	HLA match
1	M	19	CVID, SAA	Cy, Flu, ATG,	Double cord	Grade II aGVHD, (3 months)	3.83/2.73	#1 10/10 #2 10/10 Same
2 [†]	F	5	Relapsed ALL (CR2)	Mel, ATG, IT Cytarabine	Cord	None	8.6/0.96	10/10 GVHD Rej 8/10 (B,C)
3	M	15	Relapsed AML (CR2)	Mel, TBI	MRD-BM	Grade I cGVHD (6 months)	31.5/32.4	10/10 Same
4	F	17	FLT3 ITD AML (CR1)	Bu, Cy, ATG	Cord: ex vivo manipulation	Grade I aGVHD (1 & 2 months), Grade II (3 & 6 months), Grade III (12 months)	2.1/1.1	7/10 (A,Bmi,C) Same
5 [†]	F	9	Relapsed ALL (CR2)	Mel, TBI	Cord	None	10.8/4.3	9/10 (A) Same
6	M	8	Relapsed T-cell NHL (CR2)	Cy, Mel, TBI, ATG	Cord	Grade I aGVHD (3 months)	4.41/1.47	10/10 Same
7 [†]	M	13	MDS (mono 7)	Bu, Cy, Campath	MUD-BM	None	53/50.8	9/10 (A) Same
8 [†]	M	17	SAA	Cy, ATG	MRD-BM	None	64.8/34	10/10 Same
9	F	8	SCID, CHH	Mel, Flu, Campath	Cord	Grade II cGVHD (6 months)	10.2/4.6	10/10 Same
10	F	15	Relapsed AML (CR2)	Bu, Mel, ATG	Double cord	None	4.9/1.8	#1 9/10 (C) #2 8/10 (A,C) Same
11	F	11	Secondary AML (CR1), synovial sarcoma	Thiotepa, Bu, Flu	MRD-BM	None	50.1/97.2	10/10 Same
12 [†]	F	6	Sickle-cell anemia	Cy, Flu, Campath	Cord	None	4.8/0.63	7/10 (A,Bmi,C) 7/10 (A,C,DQ)
13 [†]	M	2	Osteopetrosis	Bu, Cy, ATG	MUD-PBPC	Grade IV aGVHD	7.35/4.92	9/10 (miDQ) Same
14	M	4	Epidermolysis bullosa	Bu, Flu, Campath	MRD-BM	None	51.5/90.7	10/10 Same
15 [†]	F	18	Relapsed AML with CNS/eye +)	TBI, Mel, ATG	Double cord	None	5.2/1.89	10/10 #1 9/10 (B) #2 10/10
16	M	4	WAS	Bu, Cy, ATG	Cord	None	4.97/2.6	#1 9/10 (Cmi) Same
17 [§]	M	4	AML-M7 (CR1)	Bu, Cy, ATG	Cord	None	8.63/1.75	8/10 (Bmi,C) Same
18	M	5	AML (CR2)	Bu, Cy, ATG	Cord	Grade I cGVHD (6 months)	4.67/2.61	9/10 (A) Same
19	M	17	Relapsed AML (CR2)	TBI with testicular boost, Mel	MRD-BM	None	34.3/67.2	10/10 Same
20	M	19	Relapsed AML (CR3)	Thiotepa, Bu, Flu, Campath	MUD-BM((cord?))	None	13.5/38.3	10/10 Same
21 [§]	M	5	AML (CR2)	TBI, Mel, ATG	Cord	None	6.39/4.0	10/10 Same
22	F	17	Relapsed AML (CR2)	Bu, Cy, ATG	MUD-BM	None	45.0/38.3	10/10 Same

Sex	Age	Diagnosis	Preparative regimen	Donor type	GVHD	Stem cell dose* TNC/kg (10 ⁷)/ CD34/kg (10 ⁵)	HLA match
23	M	2 Relapsed AML	TBI, Cy, Thiotepa,	Cord	Grade II aGVHD (2 months)	14.4/4.0	10/10 9/10 (Cmi)
24	M	20 Relapsed AML (CR2)	TBI with cranial radiation and testicular boost, Mel	MRD-BM	None	44.5/40.9	10/10 Same
25	M	12 High-risk ALL	TBI/Mel	MRD-BM	Grade II, aGVHD (2 months)	45.8/59.3	10/10 Same

AA, aplastic anemia; aGVHD, acute graft-vs.-host disease; ATG, antithymocyte globulin; BM, bone marrow; Bu, busulfan; cord, umbilical cord blood; CHH, cartilage-hair hypoplasia; cGVHD, chronic graft-vs.-host disease; CR3, third clinical remission; Cy, cyclophosphamide; CVID, common variable immunodeficiency; Flu, fludarabine; IT, intrathecal; MDS, myelodysplastic syndrome; Mel, melphalan; mono 7, monosomy 7; mi, minor HLA or allelic difference; MRD, matched related donor; MUD, matched unrelated donor; PBPC, peripheral blood progenitor cells; Rej, rejection; SAA, severe aplastic anemia; SCID, severe combined immunodeficiency.

* The cell doses are based upon the cryopreserved cell doses and not the actual amounts infused.

[†] Patient died.

[‡] Patient failed first transplant.

[§] Patient relapsed.

Table 2

Patient numbers and a summary of clinical characteristics

Time post-HSCT	1 month	2 months	3 months	6 months	12 months
Patient (sample size)	25	19	15	11	8
ANC (mean)	2450	3375	4350	3902	6345
G-CSF (No. of days)	6	1	0	0	0
GVHD n grade 2	1	2	2	2	1
Infections, n	8	5	5	3	0
Bacterial (n = 16)					
Fungal (n = 5)					
CSA or Tacro	100	84	78	45	25
Treatment (%)					
MMF (%)	100	53	29	9	12