Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biol Blood Marrow Transplant 20 (2014) 243–249

Low-Dose Serotherapy Improves Early Immune Reconstitution after Cord Blood Transplantation for Primary Immunodeficiencies

Jonathan P. Lane [1,2], Philippa T.G. Evans [1], Zohreh Nademi [2], Dawn Barge [2], Anthony Jackson [3], Sophie Hambleton [1,2], Terry J. Flood [2], Andrew J. Cant [1,2], Mario Abinun [1,2], Mary A. Slatter [1,2], Andrew R. Gennery [1,2,*]

[1] *Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, United Kingdom*
[2] *Newcastle upon Tyne Hospitals NHS Trust, Newcastle upon Tyne, United Kingdom*
[3] *International Centre for Life, Newcastle upon Tyne, United Kingdom*

Article history:
Received 2 April 2013
Accepted 6 November 2013

Key Words:
Primary immunodeficiency
Cord blood transplantation
Immune reconstitution
Serotherapy
Alemtuzumab

A B S T R A C T

Cord blood transplantation (CBT) is curative for many primary immunodeficiencies (PIDs) but is associated with risks of viral infection and graft-versus-host disease (GvHD). Serotherapy reduces GvHD but potentially increases the risk of viral infection by delaying immune reconstitution. Because many PID patients have pre-existing viral infections, the optimal dose of serotherapy is unclear. We performed a retrospective analysis in 34 consecutive PID patients undergoing CBT and compared immune reconstitution, viral infection, GvHD, mortality, and long-term immune function between high-dose (n = 11) and low-dose (n = 9) serotherapy. Serotherapy dose had no effect on neutrophil engraftment. Median $CD3^+$ engraftment occurred at 92.5 and 97 days for high- and low-dose serotherapy, respectively. The low-dose serotherapy group had higher $CD3^+$, $CD4^+$, and early thymic emigrant counts at 4 months compared with the high-dose group. GvHD severity and number of viral infections did not differ between serotherapy doses. Survival from the transplantation process was 90.9% for high-dose and 100% for low-dose groups. In conclusion, low-dose serotherapy enhanced T cell reconstitution and thymopoiesis during the first year after CBT with no increase in GvHD.

INTRODUCTION

Primary immunodeficiencies (PIDs) are inherited conditions characterized by recurrent infections, inflammatory disorders, and autoimmunity. Severe combined immunodeficiency (SCID), the most severe form of PID, is usually fatal within the first year of life unless corrected [1]. Other T cell and innate immune defects have excessive morbidity and mortality through childhood and early adulthood. Hematopoietic stem cell transplantation (HSCT) is curative for many patients with a 10-year survival of 84% in HLA-matched sibling HSCT for SCID [2,3]. Ten-year survival for non-SCID PID is 71% for HLA-matched sibling HSCT, with survival rates improving over time [2,3].

Umbilical cord blood offers an alternative source of stem cells for transplantation (cord blood transplantation, CBT) when a matched sibling donor is unavailable. Advantages and disadvantages of CBT over bone marrow transplantation (BMT) and peripheral blood stem cells (PBSC) are reviewed in detail elsewhere [4,5]. Advantages include (1) easy access to the cord blood unit and, therefore, earlier transplantation; (2) absence of risk to donor; (3) lower risk of latent viral transmission and graft-versus-host disease (GvHD); and (4) higher chance of matching rare HLA haplotypes. However, the stem cell dose is often low and unrelated donors are not available for boost HSCT. Cord blood units are virologically naïve and often show slower engraftment due to a lower $CD34^+$ dose compared to BMT and PBSCs. Both of these factors increase the risks from pre-existing infections in CBT for PID patients.

Evidence for the effectiveness of CBT in PID comes from a few single center and multicenter studies [6-10]. A recent study demonstrated a lower rate of grades II to IV GvHD and improved survival in unrelated CBT when the authors compared their results with similar studies of BMT for PID and that mortality was associated with pre-existing infection, no conditioning, ≥2 HLA mismatch and underlying disease [10]. However, this study does not provide data on lymphocyte reconstitution or long-term graft function. Other studies with limited data on lymphocyte reconstitution show that absolute lymphocyte counts increase from 2 months with a proportional increase in $CD4^+$ and $CD8^+$ T cells from 3 months post CBT and that age-related normal values for $CD19^+$, $CD3^+$, and $CD4^+$ are reached by 24 months in all patients studied [7,11]. All surviving patients in 1 series were independent of intravenous immunoglobulin and responded to T cell stimulation, tetanus, and hepatitis B vaccination [7].

Serotherapy in the form alemtuzumab (T and B cell depleting anti-CD52 humanized monoclonal antibody) is added to conditioning regimens to reduce the incidence of GvHD [12-16]. Immune reconstitution is delayed by alemtuzumab in patients with malignant and nonmalignant hematological conditions [13,16,17] with some studies suggesting slower immune reconstitution with higher doses used [12,18]. This slower immune reconstitution is, in turn, associated with an increased incidence of viral reactivation, notably cytomegalovirus [17, 20-22], adenovirus [14,23,24] and respiratory viruses [25]. This is particularly pertinent when considering CBT for PID because many patients have

Financial disclosure: See Acknowledgments on page 248.

* Correspondence and reprint requests: Dr Andrew R. Gennery, Department of Paediatric Immunology, Great North Children's Hospital, Royal Victoria Infirmary, Newcastle upon Tyne, UK NE1 4LP.
E-mail address: andrew.gennery@newcastle.ac.uk (A.R. Gennery).

1083-8791/$ – see front matter © 2014 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2013.11.005

active or latent viral infections at the time of HSCT and CBT is considered to have slower immune reconstitution and be virologically naïve [4,5]. The role of serotherapy in CBT for PID has not been explored to date.

We report the results of a retrospective study of the effects of 2 alemtuzumab-based serotherapy dose regimens on lymphocyte reconstitution, GvHD, viral infection, and mortality in PID patients who underwent CBT at an international center.

MATERIALS AND METHODS

Patients and Exclusion Criteria

Thirty-four consecutive patients who underwent CBT for PID between May 1999 and December 2010 at the Children's BMT Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, United Kingdom were identified from the unit's database. A retrospective study of these patients was performed, of which 14 have been previously reported [6]. There was a systematic change in serotherapy policy at the Children's BMT Unit in 2006 to 2007, when the alemtuzumab dose was reduced to determine if it had the same protective effects on GvHD without an increase in viral infection. Two patients were excluded from further analysis because they received alemtuzumab before conditioning at 3 and 4 weeks before CBT, and another was excluded because the patient received rabbit antithymocyte globulin (ATG) instead of alemtuzumb. Those who received no serotherapy (n = 11) were also excluded because 6 did not receive chemotherapeutic conditioning and others were selected to this group based on clinical presentation. The remaining patients were retrospectively nonrandomly assigned to high-dose (alemtuzumab ≥ .9 mg/kg given in 5 divided doses before day -10 or between day -8 and day -4; n = 11) or low-dose (alemtuzumab .3 to .6 mg/kg given in 3 divided doses on consecutive days between day -11 and day -6; n = 9) serotherapy groups for the purpose of analysis. No alemtuzumab pharmacokinetic studies were performed. Underlying diagnoses by serotheraphy group are shown in Supplementary Table S1.

Umbilical Cord Transplantation Procedure

Search for a suitable umbilical cord donor unit was initiated if a matched sibling cord was stored or if a matched sibling donor was unavailable. Transplantation characteristics are summarized in Table 1. HLA matching was based on low-resolution molecular class I and high-resolution molecular class II typing at 10 HLA loci as previously described [6]. Patients were conditioned according to the European Blood and Bone Marrow Transplant Group (EBMT) guidelines, EBMT Working Party for Inborn Errors, and clinical condition, and they received either myeloablative conditioning (busulphan 16 mg/kg + cyclophosphamide 200 mg/kg or treosulphan 36 g/m^2 + cyclophosphamide 200 mg/kg; n = 9) or reduced-intensity conditioning (treosulphan 36 g/m^2 + fludarabine 150 mg/m^2 or fludarabine 150 mg/m^2 + melphalan 140 mg/m^2; n = 11). Patients also received either high-dose or low-dose serotherapy as part of their conditioning regimen allocated as described above. The cord blood unit was transfused on day 0 with a median nucleated cell count of 1.30 × 10^8 cells/kg (range, .36 to 3.8) and a median CD34$^+$ count of 3.70 × 10^5 cells/kg (range, .67 to 19.0).

Table 1
Patient and Donor Cord Blood Unit Characteristics with Respect to Serotherapy Dose

	Serotherapy Dose		*P* Value
	High	Low	
No. of patients	11	9	
Year of CBT			.001
1999-2005	8	0	
2006-2010	3	9	
Age at CBT, median (range), wk	26 (8-51)	24 (7-52)	.790
Underlying diagnosis*			1.00
SCID	5	5	
Non-SCID	6	4	
Cord unit source			1.00
MUD	5	5	
MMUD	6	4	
HLA match			.496
10/10	5	5	
9/10	6	3	
8/10	0	1	
Nucleated cell dose, median (range), × 10^8/kg	1.1 (.36-3.10)	1.3 (.55-3.8)	.648
CD34$^+$ cell dose, median (range), × 10^6/kg	.29 (.067-1.45)	.63 (0.15-1.90)	.239
Chemotherapy conditioning			.406
Myeloablative	6	3	
RIC	5	6	

CBT indicates cord blood transplant; SCID, severe combined immunodeficiency; MSD, matched sibling donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; RIC, reduced-intensity conditioning.
* Further details of diagnoses are available in Supplementary Table S1.

Supportive Measures and Prophylaxis

All patients were nursed in HEPA-filtered cubicles. Patients received cyclosporine for GvHD prophylaxis, cotrimoxazole for *Pneumocystis jiroveci* prophylaxis, liposomal amphotericin for antifungal prophylaxis, and aciclovir for antiviral prophylaxis. Patients received blood and platelet support and granulocyte colony stimulating factor until engraftment, at the discretion of the managing physician. All parents gave written consent according to our local centre and European Blood and Marrow transplantation guidelines.

Immunological Studies

Time to neutrophil (third consecutive day of absolute neutrophil count (ANC) > .5 × 10^9/L), platelet (platelet count of >50 × 10^9/L independent of transfusion support) and T lymphocyte (first day of a CD3$^+$ count > 200 cells/μL) engraftment was recorded. Lymphocyte subset analysis was measured by 4-color flow cytometry as previously described [26]. Briefly, lymphocyte surface marker studies were performed on fresh whole blood collected in EDTA using appropriate markers (CD45 PerCP, CD3 FITC, CD4 APC, CD8 PE, CD19 APC, CD16/CD56 PE, CD3 PerCP/CD4 APC/CD45RAFITC/CD27 PE, CD19 PerCP/CD27 FITC/IgM APC/IgD PE [Becton Dickinson, UK Ltd, Oxford]), and analyzed on a Becton Dickinson FACS Calibur flow cytometer. The markers CD4$^+$/CD45RA$^+$ and CD4$^+$/CD45RA$^+$/CD27$^+$ were used as surrogates for early thymic emigrant equivalents (ETEEs) [27]. Lymphocyte subsets (CD3$^+$, CD4$^+$, CD8$^+$, CD19$^+$, natural killer [NK] cells and CD4$^+$ ETEEs) at 2, 4, 6, and 12 months follow-up were recorded. Specific antibody responses to pneumococcal (polysaccharide or Prevenar), tetanus toxoid and *Haemophilus influenzae* b vaccine antigens were measured by ELISA and defined as present or absent after vaccination [28].

Chimerism Studies

Whole blood was stained with CD3, CD19, or CD15 micro beads and cell lines were separated using an autoMACS automated bench-top magnetic cell sorter (Miltenyi Biotec Ltd, Surrey, UK). Chimerism was measured in sex-mismatched cases by XY-fluorescent in-situ hybridization (FISH) using standard cytogenetic techniques. Briefly, interphase FISH was performed using a Vysis 2-color CEP X/CEP Y probe set according to the manufacturer's protocol. Where donor and recipient were same sex, chimerism was measured by short tandem repeat marker analysis of genomic DNA, as previously described [29]. Most recent donor chimerism for T cell, B cell, and myeloid lineages was recorded. Chimerism was defined as donor (>95% donor cells), high-level mixed (50% to 95% donor cells), mixed (5% to 49% donor cells) or recipient (<5% donor cells) in specific cell lineages.

Viral Clearance

Patients were considered to have viral infection when a positive virology result by PCR, tissue culture, electron microscopy, or immuno-chromogenic methods was present. Viral PCR for adenovirus, cytomegalovirus, human herpes virus 6, Epstein-Barr virus, enterovirus, parainfluenza virus 3, respiratory syncytial virus, coronavirus, astrovirus, varicella-zoster virus, and norovirus; electron microscopy for small round structured virus, coronavirus, norovirus, astrovirus, and rotavirus; tissue culture for poliovirus vaccine strains; and immuno-chromogenic methods for adenovirus and rotavirus were undertaken at Newcastle Health Protection Agency Laboratories, Newcastle, United Kingdom. Viral infections were considered cleared on the first of 3 consecutively negative virology results. If there was recurrence of the same infection at a later date, this was treated as failure to clear the virus. Viral infection was considered present if the patient died with most recent result being positive or if there was still virological evidence of infection at last follow-up.

GvHD, Mortality and Follow-up

GvHD grade, target organ, and whether it was acute or chronic were recorded. GvHD was defined by modified Glucksberg criteria. Time to death and cause of death were recorded. Transplantation-related mortality (TRM) was defined as cause of death as a direct result of the transplantation procedure. Time to most recent follow-up and independence of replacement immunoglobulin were recorded.

Statistical Analysis

Differences between high- and low-dose serotherapy were analyzed. Fisher's exact and Mann-Whitney U tests were performed using SAS 9.2 for Windows (SAS Institute Inc., Cary, NC). Kaplan-Meier survival analysis, 95% confidence intervals, and log-rank test were performed using SPSS Statistics 19 (IBM SPSS, Armonk, NY). All tests were considered statistically significant if $P < .05$.

RESULTS

Transplantation Characteristics and Serotherapy Dose

Demographics and transplantation characteristics are summarized in Table 1. All patients were 12 months of age or younger at transplantation. Only year of transplantation was significantly different between high-dose and low-dose serotherapy groups ($P = .001$). Low-dose serotherapy CBT all occurred after 2006, whereas high-dose serotherapy CBT mainly occurred before 2006, consistent with the systematic change in practice.

Engraftment

Neutrophil engraftment was similar for high- and low-dose serotherapy ($P = .675$), with median engraftment occurring at 21 days (range, 13 to 38) and 20 days (range, 15 to 31), respectively. Platelet engraftment occurred at a median of 38 days (range, 18 to 114) and 31 days (range, 0 to 46) for the high- and low-dose serotherapy groups respectively ($P = .095$). Platelet engraftment took over 100 days in 1 patient in the high-dose serotherapy group.

Lymphocyte Reconstitution

$CD3^+$ lymphocyte engraftment occurred at a median time of 92.5 days (range, 56 to 222) and 97 days (range, 14 to 107) for high- and low-dose serotherapy, respectively ($P = .171$).

T lymphocyte counts increased throughout the first 12 months post CBT across the different T lymphocyte subsets (Table 2, Figure 1A-D). Reconstitution was similar at 2 months for high- and low-dose serotherapy groups for all T lymphocyte subsets. At 4 months the low-dose serotherapy group had significantly higher $CD3^+$, $CD4^+$, and $CD4^+$ ETEE counts, as compared with the high- dose group ($P = .037$, $P = .037$ and $P = .021$ respectively). $CD4^+$ ETEE reconstitution followed a similar trend to $CD4^+$ reconstitution (Figure 1D). By 6 and 12 months, $CD3^+$, $CD4^+$, and $CD4^+$ ETEE cell reconstitution in the high-dose group was similar to low-dose group. $CD8^+$ lymphocyte reconstitution was similar for high- and low-dose serotherapy groups throughout the first 12 months after CBT.

B lymphocyte counts showed a trend of increasing in number over the first 12 months post CBT for both high- and low-dose serotherapy. There was no significant difference in B cell reconstitution between high- and low-dose serotherapy groups (Table 2, Figure 1E). NK cell counts were significantly higher at 2 months ($P = .006$) for high-dose serotherapy as compared with low-dose serotherapy. At 4, 6, and 12 months, there was no significant difference in NK cell counts between serotherapy groups (Table 2, Figure 1F).

Viral Clearance

Seven patients had at least 1 viral infection at time of CBT and 7 patients contracted at least 1 viral infection post transplantation. Of these, 9 patients achieved clearance of all virus and a further 3 patients had insufficient data to determine achievement of viral clearance. There was no difference in the number of viral infections present at CBT or new viral infection after CBT between high- and low-dose serotherapy groups. All evaluable patients who received serotherapy cleared viral infections (Table 3). There were insufficient results to analyze the time to viral clearance further.

Chimerism and Graft Function

There was no significant difference in donor chimerism between high-dose and low-dose serotherapy in any of the 3 cell lineages (Figure 2). One patient with Chronic Granulomatous Disease who received high-dose serotherapy has undergone a second HSCT for falling myeloid chimerism.

All except 2 patients discontinued replacement immunoglobulin by 24 months and all those who have been vaccinated have responded to *Haemophilus influenza* b, tetanus toxoid, and pneumococcal vaccinations (Table 3). Two patients who received high-dose serotherapy were dependent on immunoglobulin therapy to 32 and 34 months and have since made a good response to primary vaccinations. One patient in the low-dose serotherapy group has

Table 2
Median Lymphocyte Counts by Month and Serotherapy Group

Lymphocyte Subset	Serotherapy Dose	Lymphocyte Count, Median (range) in Cells/μL			
		2 Months	4 Months	6 Months	12 Months
$CD3^+$	High	48 (0-515)	555 (0-1339)	1423.5 (82-3774)	3423 (158-7045)
	Low	146 (0-638)	1103 (176-1694)	1871 (203-4201)	3477 (1587-5952)
	P value	.614	*.037*	.462	.508
$CD4^+$	High	20 (0-382)	248 (0-1123)	746.5 (48-2711)	2139 (123-6077)
	Low	0 (0-519)	747 (119-1243)	1515 (176-2232)	2754 (923-4033)
	P value	.685	*.037*	.288	.085
$CD8^+$	High	46 (0-320)	143 (0-308)	304.5 (27-2821)	1071 (29-2813)
	Low	0 (0-141)	179 (0-467)	357 (49-759)	996 (415-1750)
	P value	.403	.569	.462	.965
$CD4^+$ ETEE	High	Not measured	31.5 (0-455)	318.5 (0-1691)	1614 (0-4862)
	Low	Not measured	339 (13-677)	724 (23-1318)	1786 (556-2619)
	P value	NA	*.021*	.286	.270
$CD19^+$	High	415 (0-2323)	1320 (0-4517)	1473 (32-3355)	1475 (577-2155)
	Low	1143 (405-6833)	1445 (650-2630)	1286 (278-2294)	1840 (0-2410)
	P value	.119	.382	1.00	.310
NK cells	High	357 (184-771)	485 (167-1399)	434.5 (32-1547)	288 (163-1277)
	Low	226 (136-363)	346 (93-489)	382 (125-1562)	417 (202-787)
	P value	*.006*	.113	.514	.627

Mann-Whitney U *P* values stated for difference between high- and low-dose serotherapy and considered significant if $P < .05$.

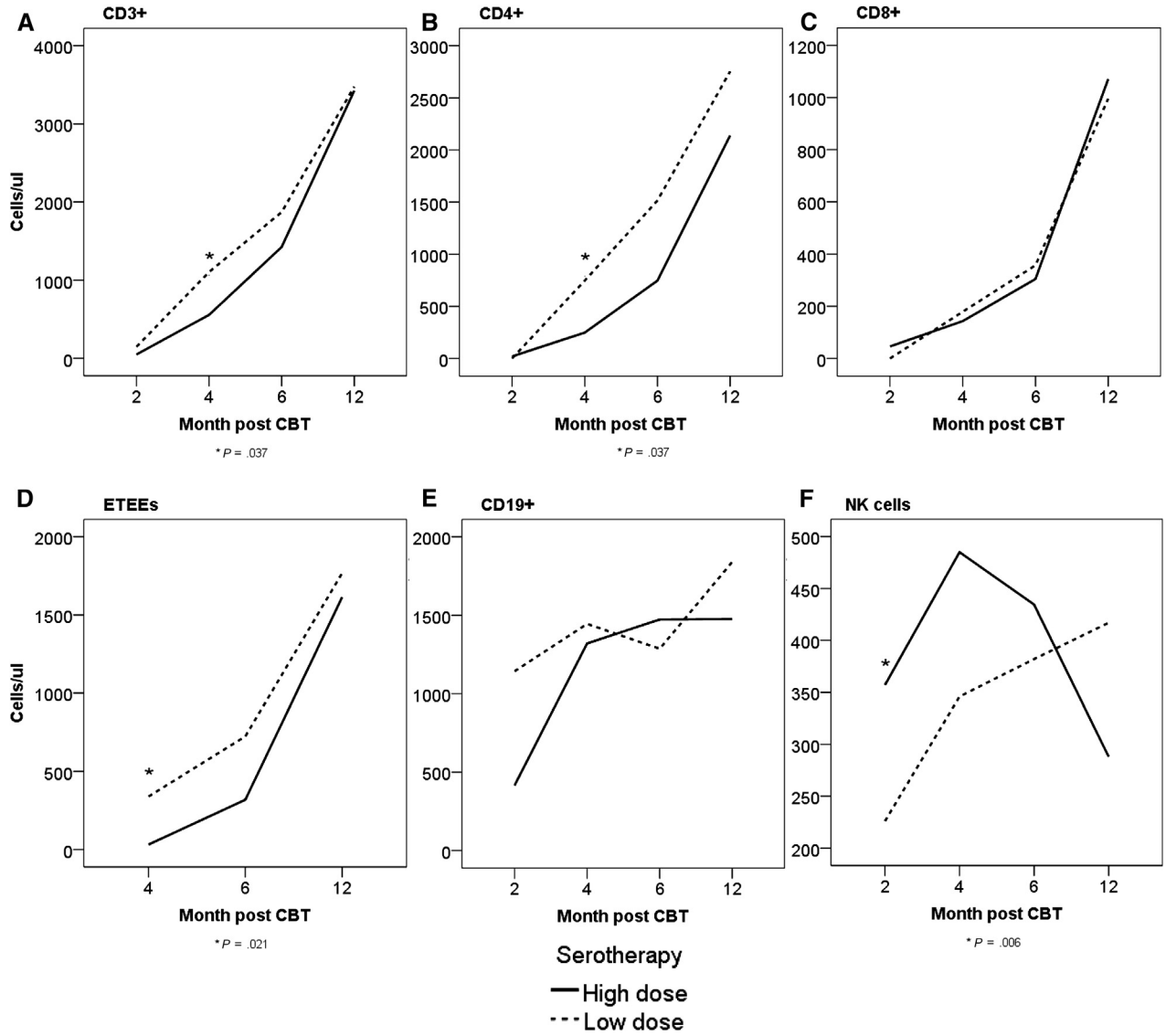

Figure 1. Immune reconstitution over the first 12 months after CBT showing median cell counts/μL for (A) $CD3^+$, (B) $CD4^+$, (C) $CD8^+$, (D) $CD4^+$ early thymic emigrant equivalents (ETEEs) (E) $CD19^+$, and (F) NK cells. *P* values given where Mann-Whitney U test showed a significant difference in cell counts between serotherapy doses.

discontinued immunoglobulin but has not yet received primary vaccinations.

GvHD and Mortality

Eight patients developed acute GvHD (40%); 5 grade II GvHD (25%). No patients experienced grade IV GvHD. There was no statistically significant difference in the severity of GvHD between high- and low-dose serotherapy groups ($P = .464$; Table 3). One patient, who received high-dose serotherapy, developed chronic skin GvHD.

All TRM occurred within 6 months of transplantation. One patient who received high-dose chemotherapy died from TRM (possible veno-occlusive disease and gastrointestinal hemorrhage complicated by chronic lung disease). Another patient who received high-dose serotherapy died from sudden infant death syndrome and was not classified as TRM. Cumulative survival from TRM in high-dose and low-dose serotherapy groups was 90.9% (95% confidence interval, 50.8% to 98.7%) and 100%, respectively, with no difference in survival between groups ($P = .366$; Figure 3).

DISCUSSION

NK and B cell reconstitution has been demonstrated to occur early after CBT, whereas low T cell numbers have been demonstrated to remain for the first 6 months post CBT [11,30], increasing the potential risk of viral infections as compared to BMT or PBSC [4,5]. Giving T lymphocyte–depleting serotherapy as GvHD prophylaxis prolongs T lymphopenia post transplantation [12,18] and giving high doses can increase the risk of viral infection further [14,17,20-25]. In our study, lower dose serotherapy improved T lymphocyte reconstitution with better thymopoiesis at 4 months post transplant than high-dose serotherapy. This is particularly pertinent in the setting of CBT for PID. In our study, the majority of patients had SCID and, therefore, further delay in T cell reconstitution from excessive serotherapy might delay clearance of pre-existing viral infection. The delay in immune reconstitution between high- and low-dose serotherapy could be explained by the prolonged exposure of the graft to lympholytic antibodies when higher doses are given. Lympholytic concentrations of alemtuzumab

Table 3
Transplantation Outcomes and Complications after Cord Blood Transplantation

	Serotherapy		*P* Value
	High Dose	Low Dose	
Viral infection			
No. patients with pre-existing infection at CBT			.404
No virus	6	8	
1 viral infection	3	1	
2 + viral infection	2	0	
No. patients with new infection post CBT			.336
No virus	8	5	
1 viral infection	3	2	
2 + viral infections	0	2	
Viral clearance			NA
No. patients achieving complete viral clearance	5	4	
No. patients not clearing all virus	0	0	
Acute GvHD			.464
0	6	6	
I	1	2	
II	4	1	
III	0	0	
Immunoglobulin replacement stopped by 24 months	7/9	9/9	NA
Achieved vaccine response	9/9	8/8	NA
Mortality	1 TRM + 1 SIDS	0	
Transplantation survival rate	.909	1.00	.366

CBT indicates cord blood transplantation; GvHD, graft-versus-host disease; TRM, transplantation-related mortality; SIDS, sudden infant death syndrome.

have been shown to be present up to 56 days after HSCT when very high doses are given in vivo [31].

A recent prospective series of 30 pediatric patients who underwent CBT for malignant and nonmalignant disease without serotherapy conditioning showed early restoration of T lymphocytes within the first 2 months post CBT from a thymic independent pathway that produced effective viral specific T cells. Thymopoiesis was evident at 1 year after transplantation [32]. In contrast to this study by Chiesa et al., our study demonstrated a delayed restoration of median T cell counts until 4 months post CBT when low-dose serotherapy was given and a further delay in T lymphocyte reconstitution to 6 months post CBT when using high dose serotherapy. $CD3^+$ reconstitution in our study improved at 4 months when low-dose serotherapy was given as compared with high-dose serotherapy, which is likely caused by corresponding improvements in $CD4^+$ and $CD4^+$ ETEE counts in the low-dose serotherapy group. The evidence of thymopoiesis in the low-dose serotherapy group at 4 months again contrasts with the study by Chiesa et al. The T cell reconstitution in the low-dose serotherapy group seen in our study at 4 months post transplantation is, therefore, likely to be explained by expansion in both a thymic dependant and independent manner.

Patients in our study experienced much lower rates of acute GvHD than in the study by Chiesa et al. (grade II to IV acute GvHD 25% versus 50%), though this may have been confounded by the inclusion of patients with malignant disease in the latter study [32]. We found the protection afforded against GvHD by lower dose serotherapy to be similar to high-dose serotherapy. In the study by Chiesa et al., 63% of patients had viral infections and 2 patients died from viral infection [32]. In our study, 35% of patients had pre-existing viral infection, and 35% developed new viral infection after CBT, with no difference between high- and low-dose serotherapy groups. This is important in the PID setting where many patients present with pre-existing viral illness. However, our definition of viral clearance and the small numbers of patients with viral infection at time of transplantation make it difficult to determine the effects of serotherapy dose on clearance of pre-existing viral infections. The influence of giving serotherapy on viral clearance in the context of PID and CBT needs further work with larger studies.

Other single and multicenter studies of CBT for PID tended to use a standard dose of ATG as serotherapy [7-10]. Knutsen et al. demonstrated that in 8 patients with T cell immunodeficiencies who received the same dose of ATG (30 mg/kg/day for 3 days) $CD4^+$ and $CD8^+$ lymphocyte

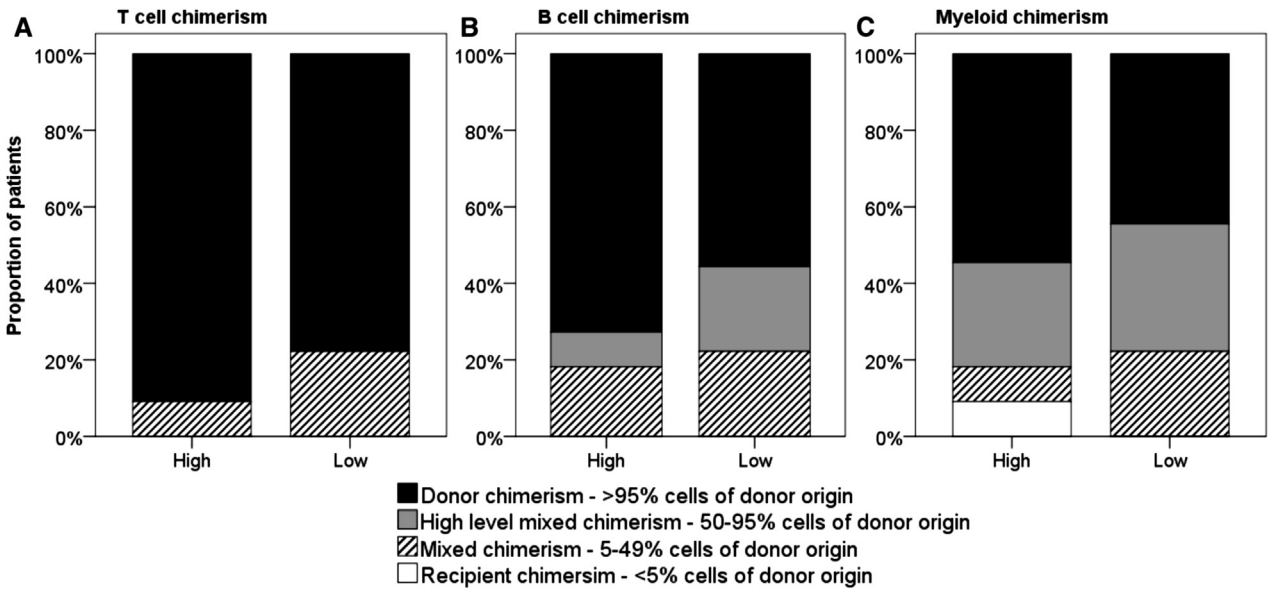

Figure 2. Most recent chimerism results for (A) T cell, (B) B cell, and (C) myeloid cell lineages for high- and low-dose serotherapy. There was no significant difference between high and low-dose serotherapy for any of cell lineage ((A) $P = .566$, (B) $P = .816$, (C) $P = .910$).

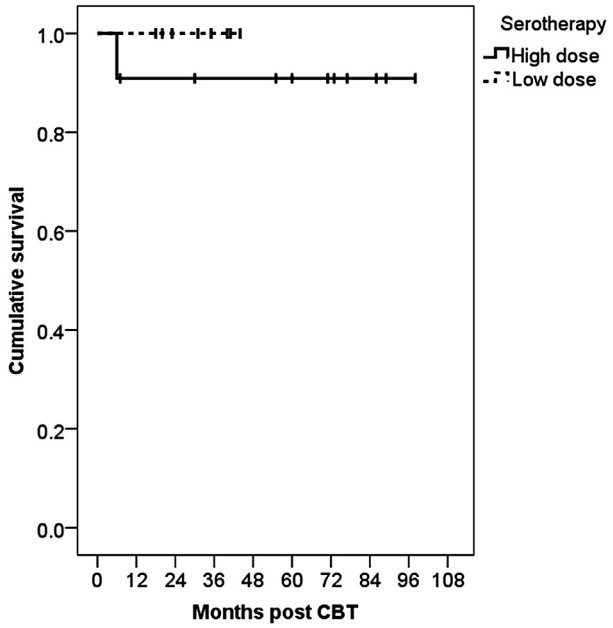

Figure 3. Kaplan-Meier survival curves representing survival from transplantation-related mortality for high dose (.909, 95% confidence interval .508-.987) and low-dose serotherapy (1.00). There was no difference in survival between high dose and low-dose serotherapy groups ($P = .366$).

numbers increased from 3 months and there was evidence of thymopoiesis (using presence of $CD4^+/CD45RA^+$ cells as marker) from 12 to 24 months [9]. This evidence of thymopoiesis occurs later than that demonstrated by the low-dose serotherapy group in our study. Overall other studies demonstrated higher rates of GvHD grades II to IV than our study [7-10]. However, these rates of GvHD are more likely to be caused by the greater number of CBT with 2 or more mismatched HLA loci, as compared with our study than the difference in serotherapy used. This, combined with the use of ATG rather than alemtuzumab, makes a direct comparison to our study difficult.

There are currently limited data regarding T cell function post CBT. Proliferative responses to mitogens and viral-specific T cell function have been demonstrated as early as 2 months post CBT [9,30,32]. The T cell receptor repertoire has been shown to have a skewed distribution at 1 year post CBT but increased diversity as compared with BMT recipients at 2 years post HSCT [33]. Our current study shows that clinically there is good long-term immune function with independence from immunoglobulin replacement by 2 years post CBT and response to primary vaccinations independent of serotherapy dose. Our study is, however, limited to looking at numerical and not functional immune reconstitution and analysis of the effects of serotherapy on T cell functional reconstitution merits further work.

Our study is limited to comparing high- and low-dose serotherapy regimens only and no comparison was made to patients not receiving serotherapy as part of their conditioning regimen. This is because patients who did not receive serotherapy were preselected based on poor clinical condition at time of transplantation, many of whom did not receive chemotherapeutic conditioning either, or they did not receive serotherapy because they received a matched sibling donor CBT. The only factor that differed significantly between the high and low-dose serotherapy groups was that low-dose serotherapy CBT occurred more recently. This was due to a systematic change in the serotherapy policy at our unit. This would have allowed for introduction of more up-to-date supportive measures in the low-dose serotherapy group, potentially influencing survival but not immune reconstitution.

Our results show that low-dose serotherapy improves speed of immune reconstitution and thymopoiesis over the first year after CBT without an increase in incidence of GvHD, as compared with high dose serotherapy. Our results also demonstrate lower rates of GvHD than in other studies. The extent that giving serotherapy affects the risk of delayed viral clearance is unclear from the current study and further research is needed.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: A.R.G. and M.A.S. designed the study; J.P.L., P.T.G.E., M.A.S., and, A.R.G. analyzed the data; J.P.L. wrote the paper; J.P.L., M.A.S., and A.R.G. were involved in reviewing the draft; Z.N., S.H., T.J.F., A.J.C., M.A., M.A.S., and A.R.G. were involved in acquisition of the data; and D.B. and A.J. provided laboratory tests.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.11.005.

REFERENCES

1. van der Burg M, Gennery AR. Educational paper. The expanding clinical and immunological spectrum of severe combined immunodeficiency. *Eur J Pediatr*. 2011;170:561-571.
2. Antoine C, Muller S, Cant A, et al. Long-term survival and transplantation of haemopoietic stem cells for immunodeficiencies: report of the European experience 1968-99. *Lancet*. 2003;361:553-560.
3. Gennery AR, Slatter MA, Grandin L, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: Entering a new century, do we do better? *J Allergy Clin Immunol*. 2010;126:602-610.
4. Gennery AR, Cant AJ. Cord blood stem cell transplantation in primary immune deficiencies. *Curr Opin Allergy Clin Immunol*. 2007;7:528-534.
5. Slatter MA, Gennery AR. Umbilical cord stem cell transplantation for primary immunodeficiencies. *Expert Opin Biol Ther*. 2006;6:555-565.
6. Bhattacharya A, Slatter MA, Chapman CE, et al. Single centre experience of umbilical cord stem cell transplantation for primary immunodeficiency. *Bone Marrow Transplant*. 2005;36:295-299.
7. de Heredia CD, Ortega JJ, Diaz M, et al. Unrelated cord blood transplantation for severe combined immunodeficiency and other primary immunodeficiencies. *Bone Marrow Transplan*. 2008;41:627-633.
8. Frangoul H, Wang L, Harrell FE, et al. Unrelated umbilical cord blood transplantation in children with immune deficiency: results of a multicenter study. *Bone Marrow Transplant*. 2010;45:283-288.
9. Knutsen AP, Wall DA. Umbilical cord blood transplantation in severe T-cell immunodeficiency disorders: Two-year experience. *J Clin Immunol*. 2000;20:466-476.
10. Morio T, Atsuta Y, Tomizawa D, et al. Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan. *Br J Haematol*. 2011;154:363-372.
11. Knutsen AP, Wall DA. Kinetics of T-cell development of umbilical cord blood transplantation in severe T-cell immunodeficiency disorders. *J Allergy Clin Immunol*. 1999;103:823-832.
12. Chakraverty R, Orti G, Roughton M, et al. Impact of in vivo alemtuzumab dose before reduced intensity conditioning and HLA-identical sibling stem cell transplantation: pharmacokinetics, GVHD, and immune reconstitution. *Blood*. 2010;116:3080-3088.
13. Dodero A, Carrabba M, Milani R, et al. Reduced-intensity conditioning containing low-dose alemtuzumab before allogeneic peripheral blood stem cell transplantation: graft-versus-host disease is decreased but T-cell reconstitution is delayed. *Exp Hematol*. 2005;33:920-927.
14. Myers GD, Krance RA, Weiss H, et al. Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). *Bone Marrow Transplant*. 2005;36:1001-1008.

15. Poire X, van Besien K. Alemtuzumab in allogeneic hematopoetic stem cell transplantation. *Expert Opinion on Biological Therapy*. 2011;11:1099-1111.
16. Shah AJ, Kapoor N, Crooks GM, et al. The effects of Campath 1H upon graft-versus-host disease, infection, relapse, and immune reconstitution in recipients of pediatric unrelated transplants. *Biol Blood Marrow Transplant*. 2007;13:584-593.
17. Chakrabarti S, Mackinnon S, Chopra R, et al. High incidence of cytomegalovirus infection after nonmyeloablative stem cell transplantation: potential role of Campath-1H in delaying immune reconstitution. *Blood*. 2002;99:4357-4363.
18. Juliusson G, Theorin N, Karlsson K, et al. Subcutaneous alemtuzumab vs ATG in adjusted conditioning for allogeneic transplantation: influence of Campath dose on lymphoid recovery, mixed chimerism and survival. *Bone Marrow Transplant*. 2006;37:503-510.
19. Landgren O, Gilbert ES, Rizzo JD, et al. Risk factors for lymphoproliferative disorders after allogeneic hematopoietic cell transplantation. *Blood*. 2009;113:4992-5001.
20. Bainton RD, Byrne JL, Davy BJ, Russell NH. CMV infection following nonmyeloablative allogeneic stem cell transplantation using Campath. *Blood*. 2002;100:3843-3844.
21. Buyck HC, Prentice HGF, Griffiths PD, Emery VC. The risk of early and late CMV DNAemia associated with Campath use in stem cell transplant recipients. *Bone Marrow Transplant*. 2010;45:1212-1219.
22. Lamba R, Carrum G, Myers GD, et al. Cytomegalovirus (CMV) infections and CMV-specific cellular immune reconstitution following reduced intensity conditioning allogeneic stem cell transplantation with alemtuzumab. *Bone Marrow Transplant*. 2005;36:797-802.
23. Avivi I, Chakrabarti S, Milligan DW, et al. Incidence and outcome of Adenovirus disease in transplant recipients after reduced-intensity conditioning with alemtuzumab. *Biol Blood Marrow Transplant*. 2004;10:186-194.
24. Chakrabarti S, Mautner V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood*. 2002;100:1619-1627.
25. Chakrabarti S, Avivi I, Mackinnon S, et al. Respiratory virus infections in transplant recipients after reduced-intensity conditioning with Campath-1H: high incidence but low mortality. *Br J Haematol*. 2002;119:1125-1132.
26. Berrington JE, Barge D, Fenton AC, et al. Lymphocyte subsets in term and significantly preterm UK infants in the first year of life analysed by single platform flow cytometry. *Clin Exp Immunol*. 2005;140:289-292.
27. Steffens CM, Managlia EZ, Landay A, Al-Harthi L. Interleukin-7-treated naive T cells can be productively infected by T-cell-adapted and primary isolates of human immunodeficiency virus 1. *Blood*. 2002;99:3310-3318.
28. Gennery AR, Cant AJ, Spickett GP, et al. Effect of immunosuppression after cardiac transplantation in early childhood on antibody response to polysaccharide antigen. *Lancet*. 1998;351:1778-1781.
29. Routledge D, Jackson A, Bourn D, et al. Quantitative assessment of mixed chimerism in allogeneic stem cell transplant patients - A comparison of molecular genetic and cytogenetic approaches. *J Pediat Hematol Onc*. 2007;29:428-431.
30. Komanduri KV, John LSS, de Lima M, et al. Delayed immune reconstitution after cord blood transplantation is characterized by impaired thymopoiesis and late memory T-cell skewing. *Blood*. 2007;110:4543-4551.
31. Morris EC, Rebello P, Thomson KJ, et al. Pharmacokinetics of alemtuzumab used for in vivo and in vitro T-cell depletion in allogeneic transplantations: relevance for early adoptive immunotherapy and infectious complications. *Blood*. 2003;102:404-406.
32. Chiesa R, Gilmour K, Qasim W, et al. Omission of in vivo T-cell depletion promotes rapid expansion of naïve CD4+ cord blood lymphocytes and restores adaptive immunity within 2 months after unrelated cord blood transplant. *Br J Haematol*. 2012;156:656-666.
33. Talvensarri K, Clave E, Douay C, et al. A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. *Blood*. 2002;99:1458-1464.