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Effects of Conditioning Regimens and T Cell Depletion in Hematopoietic Cell Transplantation for Primary Immune Deficiency

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This study analyzes the hematopoietic cell transplantation experience in patients with immune deficiency at a single institution. The objective is to comprehensively evaluate the short-term and long-term outcomes with various preparative regimens, donor grafts, and ex vivo manipulations to identify transplantation approaches that most likely favor early donor immune competency without generating excessive toxicity. Clinical outcomes were evaluated in 52 consecutive patients with immune deficiencies. Thirty-seven of the 52 patients (71%) survived with attenuation of their underlying disease. The use of a melphalan-based reduced-intensity conditioning preparative regimen and immunomagnetic $CD3^+$ T cell depletion techniques (when T cell depletion was indicated) were associated with improved event-free survival. Survivors who received a preparative regimen other than a melphalan-based reduced-intensity regimen suffered from therapy-related morbidities or chronic/recurrent infections. Our findings indicate that melphalan-based reduced-intensity conditioning regimens and immunomagnetic $CD3^+$ T cell depletion limit therapy-related toxicity, and demonstrate promising results for the early establishment of donor immune competency.

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INTRODUCTION

Since the earliest hematopoietic cell transplantations (HCT) performed more than 40 years ago, HCT has been a curative option for several primary immune deficiencies, including severe combined immune deficiency (SCID) and Wiskott-Aldrich syndrome (WAS) [1,2]. Although gene therapy is a promising future alternative, HCT remains the therapy of choice for the majority of patients [3,4]. However, despite decades of experience with HCT for these diseases, the optimum strategies for successful transplantation continue to evolve with time, and remain controversial.

The history of HCT for SCID illustrates this evolution. It is particularly successful in patients with SCID, with >80% long-term survival when an HLA-matched sibling donor is available [5]. Even when alternative donors are required, the majority of recipients survive [5-7]. The success of HCT in patients with SCID is due in large part to a significantly reduced immune barrier, allowing minimal or no conditioning before transplantation [6]. Although the earliest HCTs for SCID typically used conventional myeloablative techniques, it became clear that avoiding myeloablative conditioning regimens was desirable to reduce transplantation-related mortality. The preference then shifted toward performing even mismatched donor HCT in patients with SCID who received conditioning without chemotherapy or radiation therapy after this type of HCT was found to be well tolerated and largely successful [6]. However, recent results indicate that the lack of myeloablation can lead to incomplete long-term donor immune reconstitution and untoward clinical outcomes [8-10]. When myeloablative preparative regimens are used, complete replacement of the defective immune system is more readily achieved [8], but at a cost of possibly increased

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mortality and short- and long-term morbidity [11]. This has led many institutions, including ours, to use reduced-intensity conditioning (RIC) regimens to minimize toxicity and deliver fully competent long-term donor engraftment. Although RIC regimens may deliver reduced toxicity, many current RIC regimens fail to achieve fully competent long-term donor immune reconstitution [8,12,13].

In contrast to SCID, other immune deficiencies, such as WAS, have a significant host immunologic barrier to overcome. They do, however, share the same desired outcome of minimal toxicity with long-term donor immunocompetency [14]. Here we describe the experience with allogeneic HCT for immune deficiencies at a single institution, evaluating both short-term and long-term outcomes for the evolving therapies in this patient population, and identify potential strategies for future patients.

PATIENTS AND METHODS

Patients

This study was a retrospective analysis of all HCTs performed in patients with immune deficiency between 1991 and 2010 at St. Jude Children's Research Hospital. A written protocol was submitted to, and approval was obtained from, St. Jude's Institutional Review Board before the initiation of research activities. Data were collected and analyzed in anonymized fashion, in accordance with the rules and regulations of the Health Information Portability and Accountability Act and the Institutional Review Board.

Fifty-nine consecutive patients were identified. Six patients with X-linked agammaglobulinemia who received a sibling graft with no conditioning were described previously [15] and were excluded from the present analysis. One patient who underwent a first HCT at an outside institution was also excluded, leaving 52 patients, who received a total of 70 HCTs, for analysis. This cohort includes 23 patients with SCID, 10 with WAS, 9 with familial hemophagocytic lymphohistiocytosis (HLH), 4 with X-linked lymphoproliferative syndrome (XLP), 2 with CD40-ligand deficiency (CD40L), 1 with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX), 1 with leukocyte adhesion deficiency (LAD) type 1, 1 with chronic granulomatous disease, and 1 with Chediak-Higashi syndrome (CHS). Twenty-five patients received their initial transplants from parental donors (18 matched at 3 of 6 loci, 6 matched at 4 of 6 loci, and 1 a phenotypic 6 of 6 match), 16 patients received their initial transplants from matched unrelated donors (3 matched at 5 of 6 loci and 13 matched at a minimum of 6 of 6 loci), and 11 patients received their initial transplants from genotypically matched sibling donors (Table 1).

Methods

All patients were housed in high-efficiency particulate air–filtered rooms on an HCT unit during the peritransplantation period. Standard supportive care guidelines were followed for all patients and typically included pneumocystis prophylaxis with trimethoprim-sulfamethoxazol, antifungal prophylaxis with an azole or echinocandin, and anti-herpes simplex virus and cytomegalovirus (CMV) prophylaxis with acyclovir. Weekly monitoring for Epstein-Barr virus (EBV), CMV, and adenovirus by quantitative PCR and for aspergillosis by galactamanin assay was instituted in 2002. Before 2000, all parental donor recipients received marrow grafts (n = 10); later recipients received peripheral blood stem cells (PBSCs) (n = 15). All sibling and unrelated donor transplant recipients received marrow grafts, except 2 unrelated donor transplant recipients with WAS who received PBSCs.

All 25 primary parental grafts were T cell depleted (TCD). Seven of 16 unrelated grafts, and none of the 11 sibling grafts, were TCD. Before 2001, grafts were rendered TCD by complement mediated lysis with either CD3 or CD6/CD8 antibodies (complement/Ab) (n = 16). Before 2001, grafts were rendered TCD using the CliniMACS device (n = 16) initially via $CD34^+$ cell selection (n = 5) and later via $CD3^+$ cell depletion (n = 11). Median $CD3^+$ cell doses for TCD grafts were 1.02×10^6/kg for complement/Ab, 0.01×10^6/kg for CliniMACS $CD34^+$ selection, and 0.01×10^6/kg for CliniMACS $CD3^+$ depletion, compared with 33.11×10^6/kg for unmanipulated grafts. Median $CD34^+$ cell doses for TCD grafts were 2.77×10^6/kg for complement/Ab, 41.59×10^6/kg for CliniMACS $CD34^+$ selection, and 27.74×10^6/kg for CliniMACS $CD3^+$ depletion, compared with 7.24×10^6/kg for unmanipulated grafts.

Through 2005, preparative regimens were primarily myeloablative (busulfan or total body irradiation [TBI]) or used no chemotherapy or radiotherapy. Five patients received a TBI dose of 1200 cGy, with 1140 cGy given to 1 patient and 1400 cGy given to 1 patient. Busulfan dosing was i.v. and targeted in one-half of the patients (12 of 24), with the rest receiving oral dosing. Dosing was myeloablative in all busulfan recipients, with a median cumulative busulfan dose of 16 mg/kg. Between 2006 and 2010, 10 of 13 patients received melphalan-based RIC ($\leq$140 mg/m^2).

The choices of T cell depletion technique and conditioning regimen were based on the protocol available or physician discretion. An HLA-matched sibling was the preferred donor for all patients when available. An HLA-matched unrelated donor was the second choice for non-SCID recipients. Parental donors were used in non-SCID recipients only when no matched (related or unrelated) donor was available,

Table 1. Patients

	Total (n = 52)	SCID (n = 23)	WAS (n = 10)	HLH (n = 9)	Other (n = 10)
Age, years, median (range)	0.8 (0.0-18.9)	0.5 (0.0-0.9)	4.7 (1.5-10.9)	1.3 (0.5-4.1)	8.6 (0.7-18.9)
Donor type, n (%)					
Parent	25 (48)	19 (83)	2 (20)	3 (33)	1 (10)
Sibling	11 (21)	4 (17)	2 (20)	2 (22)	3 (30)
Unrelated	16 (31)	0 (0)	6 (60)	4 (44)	6 (60)
Preparative regimen, n (%)					
Myeloablative	31 (60)	9 (39)	8 (80)	8 (89)	6 (60)
Bu + Cy-based	22	9	4	4	5
Flu + Bu-based	2			2	
TBI + Cy-based	7		4	2	1
Reduced intensity	10 (19)	3 (13)	2 (20)	1 (11)	4 (40)
Flu + Mel-based	9	2	2	1	4
Mel	1	1			
No chemotherapy	11 (21)	11 (48)	0 (0)	0 (0)	0 (0)
Cyclosporine, n (%)					
No	25 (48)	17 (74)	2 (20)	3 (33)	3 (30)
Yes	27 (52)	6 (26)	8 (80)	6 (67)	7 (70)
Anti–T cell antibody, n (%)					
No	23 (44)	16 (70)	1 (10)	3 (33)	3 (30)
Yes	29 (56)	7 (30)	9 (90)	6 (67)	7 (70)
TCD, n (%)					
Antibody/complement	16 (31)	10 (43)	4 (40)	2 (22)	0 (0)
CliniMACS	16 (31)	9 (39)	2 (20)	3 (33)	2 (20)
None	20 (38)	4 (17)	4 (40)	4 (44)	8 (80)
HLA match status (n of 6), n (%)					
3	18 (35)	13 (57)	1 (10)	3 (33)	1 (10)
4	6 (12)	5 (22)	1 (10)	0 (0)	0 (0)
5	3 (6)	0 (0)	3 (30)	0 (0)	0 (0)
6	25 (48)	5 (22)	5 (50)	6 (67)	9 (90)
Maximum acute GVHD grade, n (%)					
0	36 (69)	20 (87)	5 (50)	5 (56)	6 (60)
I	7 (13)	2 (9)	2 (20)	2 (22)	1 (10)
II	5 (10)	1 (4)	1 (10)	1 (11)	2 (20)
III	3 (6)	0 (0)	2 (20)	1 (11)	0 (0)
IV	1 (2)	0 (0)	0 (0)	0 (0)	1 (10)

and preferentially in SCID recipients with no HLA-matched sibling donor.

Before 2000, donor chimerism was determined by fluorescent in situ hybridization analysis, available predominantly in bone marrow specimens in sex-mismatched patients. Later, donor chimerism was always available, predominantly in peripheral blood samples, using variable number tandem repeat analysis. Lineage-specific chimerism was not performed routinely.

Statistical Analysis

The evaluated clinical outcomes included overall survival (OS) and event-free survival (EFS). OS was defined as the time from the date of first HCT to death from any cause, and EFS was defined as the time from the date of first HCT to the occurrence of death from any cause or graft failure. Graft failure was defined as <5% donor chimerism or failure of donor immune reconstitution necessitating a second HCT. The decision to proceed to a second HCT was made jointly by immunology and transplant clinicians. Patients who were still alive without experiencing an event at their last follow-up date were considered censored in survival estimates.

OS and EFS distributions were estimated by the Kaplan-Meier method [16] and compared using the exact log-rank test [17]. The Fisher exact test, χ^2 test, and Kruskal-Wallis test were used to compare categorical variables across groups. $P < .05$ was the nominal significance level. SAS version 9.2 (SAS Institute, Cary, NC) and StatXact Windows version 8 (Cytel, Cambridge, MA) were used for statistical analysis.

RESULTS

SCID

All 23 patients with SCID received a related donor graft (Table 1). Four patients received a sibling graft; all were alive and clinically well at last follow-up. The remaining 19 patients received a parental graft. Three of these patients died within 90 days of first HCT, and 2 others died shortly after a second HCT. Four of the 5 patients who died succumbed to documented invasive infection (2 with *aspergillosis*, 1 with CMV, and 1 with respiratory syncytial virus), and 1 died of rapid pulmonary failure (no organism identified) (Table 2). Fourteen patients survived a median of 7.5 years after parental donor HCT, for a 6-year OS of

Table 2. Snapshot of Clinical Outcomes

UPN	Donor	Preparation	Died, Day	Alive, Age, Years	Disease Type	Disease Phenotype	Outcome Summary
SCID patients							
1	Sibling	BuCy		12.0	RAG	B^-NK^+	Clinically well; no medications
2	Sibling	BuCy		15.3	AR	NA	Clinically well; playing soccer, good in school
3	Sibling	Mel		2.2	RAG	B^-NK^+	Clinically well; off IVIG, getting vaccinations
4	Sibling	No		0.2	AR	B^+NK^+	Lost to follow-up on return to home institution
5	Haploidentical	BuCy		15.7	XL	B^+NK^-	On augmentin, Zithromax, IVIG; chronic cough and chronic stable bronchiectasis
6	Haploidentical	BuCy	20		ADA	B^-NK^-	MOF, *Aspergillosis*
7	Haploidentical	BuCy		13.0	XL	B^+NK^-	Well, in school; mild intermittent asthma
8	Haploidentical	BuCy		11.4	XL	B^+NK^-	Well, in school; hypothyroid on synthroid, osteoporosis
9	Haploidentical	BuCy		10.7	RAG	B^-NK^+	Well, in school; on SQIG and Septra, chronic neutropenia and thrombocytopenia
10	Haploidentical	BuCy		18.2	XL	NA	Chronic warts, chronic cough; smokes, noncompliant with augmentin
11	Haploidentical	BuCy		3.4	CHHS	NA	Clinically well, in school; dwarfed owing to cartilage-hair hypoplasia syndrome
12	Haploidentical	No	24		NA	B^+NK^-	ARDS, biopsy showed interstitial necrotizing alveolitis, cultures negative
13	Haploidentical	No		13.6	XL	B^+NK^-	Clinically well; recurrent warts, on IVIG and Bactrim
14	Haploidentical	No	1097		ADA	B^-NK^-	*Aspergillosis*, ARDS, acute renal failure
15	Haploidentical	No		7.9	γc	B^+NK^-	Post-HCT RSV; now clinically well, limited skin chronic GVHD, on Bactrim and IVIG
16	Haploidentical	No		7.0	γc	B^+NK^-	Clinically well; intermittent sinopulmonary infections, on IVIG and Bactrim
17	Haploidentical	No*		6.4	NA	NA	In school; nephogenic diapetes insipidus, juvenile rheumatoid arthritis on methotrexate, on Bactrim
18	Haploidentical	No		1.9	ADA	B^-NK^-	Tracheostomy due to pre-HCT pseudomonas, on SQIG and Bactrim
19	Haploidentical	No	240		CD3δ	B^+NK^+	ARDS, pulmonary failure, RSV pneumonitis, adenovirus in blood and stool
20	Haploidentical	No	86		AR	B^-NK^+	ARDS, CMV pneumonitis, *Pseudomonas* sepsis
21	Haploidentical	No*		3.6	ADA	B^-NK^-	On ADA replacement, CMV reactivation now resolved, on IVIG and Bactrim
22	Haploidentical	FluMel		1.2	IL7R	B^+NK^+	*Mycobacterium* infection of hand resolved; growing and developing
23	Haploidentical	FluMel		1.2	IL7R	B^+NK^+	Well; growing and developing appropriately
WAS patients							
24	Sibling	BuCy		8.5	*Bacillus cereus* bacteremia, fungus on scan; now clinically well, HTN on lisinipril		
25	Sibling	BuCy		15.2	Preparing for college; chronic GVHD skin, on Cellcept, topical tacrolimus, Bactrim, and penVK		
26	Unrelated	BuCy		7.3	Coagulase-negative *Staphylococcus* bacteremia; now clinically well		
27	Unrelated	BuCy	84		MOF, CMV, EBV, and adenovirus in blood		
28	Unrelated	TBI	152		EBV PTLD, CMV in blood, ARDS, pulmonary failure, *Staphylococcus aureus* bacteremia		
29	Unrelated	TBI		11.0	Clinically well; swimming and surfing, wrestling		
30	Unrelated	TBI		10.0	Post-HCT cellulitis, urinary tract infection; now attention deficit hyperactivity disorder, on Synthroid, penVK postsplenectomy		
31	Unrelated	TBI	250		*Enterococcus*, *S aureus*, *Candida* in blood, pulmonary *Aspergillosis*, EBV PTLD		
32	Haploidentical	FluMel		4.3	Post-HCT *Clostridium difficile*, CMV and EBV reactivation; now clinically well, no medications		
33	Haploidentical	FluMel		4.2	Post-HCT RSV, *S aureus* bacteremia; now clinically well, myringotomy tubes now out		
HLH patients							
34	Sibling	TBI		12.1	Short stature, osteochondrma		
35	Sibling	BuCY	38		Hepatic VOD, MOF		
36	Unrelated	BuCY		13.5	On GH and Synthroid; hypertension, HTN on amlodipine, renal insufficiency on erythropoietin, ovarian failure on estrogen		
37	Unrelated	BuCY		7.0	Post-HCT EBV reactivation, systemic inflammatory response syndrome; now feels well, rapid puberty, on Lupron		
38	Unrelated	BuCY		6.0	Intubated for respiratory insufficiency; now feels well, metabolic syndrome, no medications		
39	Unrelated	TBI		9.1	Feels well; good in school, on GH and Sythroid, cataract removal, osteochondroma		
40	Haploidentical	FluBu	226		Adenoviral pneumonia, *Aspergillosis*		
41	Haploidentical	FluBu	28		Progressive EBV		
42	Haploidentical	FluMel	76		Bowel perforation, MOF, *Enterococcus*, *Pseudomonas*, *Aspergillosis*, parainfluenza.		
XLP patients							
43	Unrelated	BuCY		5.5	Early *Pseudomonas* sepsis, CMV, BK virus; now worked up for depression, intermittent sinusitis		
44	Unrelated	FluMel		2.5	Burkitt lymphoma and short gut pre-HCT, EBV reactivation post-HCT resolved; otherwise well		

(*Continued*)

Table 2. (*Continued*)

UPN	Donor	Preparation	Died, Day	Alive, Age, Years	Disease Type	Disease Phenotype	Outcome Summary
45	Unrelated	TBI	182		Interstitial pneumonitis, respiratory failure, EBV		
46	Haploidentical	BuCY	41		Hepatic VOD, MOF, adenovirus		
Other patients							
47	Sibling	BuCy		9.6	40L		Clinically well, only medication is Synthroid
48	Sibling	BuCy		4.0	40L		GVHD resolved, active; mild osteoporosis and hypercholersterolemia
49	Sibling	BuCY		13.0	CHS		Neurocognitive difficulty; insulin resistance syndrome, on metformin
50	Unrelated	FluMel		2.0	LAD1		EBV reactivation, LAD corrected; clinically well, DLI-induced limited skin GVHD resolved
51	Unrelated	FluMel		2.6	CGD		Post-HCT EBV reactivation, seizure; now well, in school, mild osteoporosis improving
52	Unrelated	FluMel		3.6	IPEX		Early *S aureus* bacteremia, adenoviral and *C difficile* colitis; now no problems

40L indicates CD40 ligand deficiency; ADA, adenosine deaminase deficiency; AR, autosomal recessive; ARDS, acute respiratory distress syndrome; CD3δ, CD3δ deficiency; CDG, chronic granulomatous disease; FluMel, fludarabine + melphalan-based regimen; γc, common γ-chain deficiency; GH, growth hormone; HTN, hypertension; IL7R, interleukin-7 receptor deficiency; mel, melphalan + campath only; MOF, multiorgan failure; NA, not available; PTLD, posttransplantation lymphoproliferative disorder; RAG, RAG deficiency; RSV, respiratory syncytial virus; sibling, matched sibling donor; SQIG, s.c. immune globulin; TBI, total body irradiation + cyclophosphamide-based regimen; unrelated, matched unrelated donor; VOD, veno-occlusive disease; XL, X-linked.
*Campath alone.

72% ± 12%. The 6-year OS of the entire cohort of SCID patients was 76% ± 11%. The small number of patients precludes subset analysis, but of note, 7 of 7 X-linked SCID recipients survived.

Falling donor chimerism and overt graft failure was a significant problem for early parental donor recipients regardless of whether they received busulfan plus cyclophosphamide (BuCy) or no conditioning. Only 3 of the first 17 recipients achieved donor engraftment without the need for subsequent HCT. Three patients died in the early posttransplantation period, 1 patient had no donor chimerism and was maintained on enzyme replacement, and the 10 remaining recipients received a total of 26 HCTs. A majority of subsequent transplants were from the previous donor without conditioning, but 9 of the 10 patients who underwent subsequent HCT received conditioning with at least 1 of their transplants. Eight of these 10 patients survived long-term.

The 2 most recent parental donor recipients received a melphalan-based RIC regimen with fludarabine and alemtuzumab. Both patients received maternal PBSC grafts that had been rendered TCD by CliniMACS $CD3^+$ depletion and received low-dose donor lymphocyte infusion (DLI; 0.05×10^6 $CD3^+$ cells/kg) electively at 12 weeks posttransplantation before returning home. At the time of this report, both patients are well and maintain full donor T cell chimerism.

None of the 17 SCID survivors followed at our institution achieved full donor chimerism (Table 3). All 3 sibling recipients are i.v. immunoglobulin (IVIG)-independent. However, 7 of 14 parental donor recipients remain on IVIG replacement, including 2 of 6 patients conditioned with BuCy and 5 of 6 patients who initially received no conditioning. Although the few sibling recipients are uniformly well, long-term antibiotic use is necessary in most parental donor recipients, who are susceptible to intermittent and chronic infections (Table 2).

WAS

Two patients with WAS received a sibling graft, 6 received an unrelated graft, and 2 received a parental donor graft (Table 1). Seven of the 10 patients survive, at a median of 8.7 years post-HCT (Table 2). All 6 unrelated donor transplant recipients received myeloablative conditioning. Two developed grade III GVHD and subsequently died of infectious etiologies. Both were age >8 years at the time of HCT, and both received a TCD unrelated donor graft with a TBI-based preparative regimen. Another patient died during the first year with viral infection.

Both sibling donor transplant recipients received BuCy conditioning and currently survive. One developed grade II acute GVHD but is clinically well more than 15 years post-HCT despite some continued limited skin chronic GVHD. The 2 patients with WAS who received a parental donor HCT have survived more than 4 years post-HCT. They received conditioning with fludarabine, thiotepa, melphalan, and OKT3. The grafts were $CD3^+$ TCD with CliniMACS. Both patients are clinically well with full donor chimerism, no GVHD, and no IVIG replacement therapy (Table 3). One patient has a low level of IgA, but normal IgG and IgM levels. Both patients have documented protective titers to polio, hepatitis B, mumps, and diphtheria after vaccination (Supplemental Table). Currently, 6 of 7 WAS survivors have full donor chimerism, and none have developed autoimmune disease.

Table 3. Immune Reconstitution in Survivors at Last Follow-Up

UPN	Donor	Conditioning	TCD	Year	HCT, n	Chimerism (Donor)	IVIG	IgG	IgM	IgA	Lymphocytes, × 10^6/uL Total	$CD3^+$	$CD3^+CD4^+$	$CD3^+CD8^+$	$CD56^+$	$CD19^+$
SCID Survivors																
1	Sibling	BuCy	No	12.0	1	NA	Off	1334	69	276	1600	1020	570	390	340	**40**
2	Sibling	BuCy	No	15.3	1	NA	Off	721	254	106	NA					
3	Sibling	Mel	No	2.2	1	27%	Off	770	128	41	1500	1100	**390**	530	350	**50**
5	Haploidentical	BuCy	Ab	15.7	1	B cell; 2%	Still on	882	**28**	**<4**	800	**780**	**150**	580	**<10**	**20**
7	Haploidentical	BuCy	Ab	13.0	2	NA	Off	1066	122	98	3500	2770	1230	1470	210	560
8	Haploidentical	BuCy	Ab	11.4	2	NA	Off	868	120	235	1600	1410	560	720	**80**	110
9	Haploidentical	BuCy	Ab	10.7	3	23%	Still on	726	**30**	74	400	**330**	**240**	**90**	**60**	**10**
10	Haploidentical	BuCy	Ab	18.2	2	B cell; 1%	Off	**304**	60	**23**	1100	1050	**460**	610	**30**	**20**
11	Haploidentical	BuCy	Ab	3.4	3	52%	Off	873	104	193	NA					
13	Haploidentical	No	Ab	13.6	4	NA	Still on	951	**44**	119	2800	2520	980	1230	**80**	**20**
15	Haploidentical	No	34	7.9	2	38%	Still on	881	**26**	**<4**	2900	2730	1100	1560	**30**	140
16	Haploidentical	No	3	7.0	1	16%	Still on	**497**	40	**11**	2600	2440	600	1270	**<10**	160
17	Haploidentical	No*	3	6.4	3	20%	Off	1006	55	39	700	**480**	**270**	**180**	150	**70**
18	Haploidentical	No	3	1.9	1	37%	Still on	854	**28**	34	2200	1970	1250	440	60	110
21	Haploidentical	No*	3	3.6	1	Absent	Still on	905	84	37	1500	**820**	**80**	680	630	110
22	Haploidentical	FluMel	3	1.2	1	45%	Off	694	70	**8**	2900	2090	1330	730	410	410
23	Haploidentical	FluMel	3	1.2	1	35%	Off	490	**44**	**<4**	1200	980	720	230	**70**	160
WAS survivors																
24	Sibling	BuCy	No	8.5	1	Full	Off	845	66	87	1600	980	580	300	110	500
25	Sibling	BuCy	No	15.2	1	Full	Off	919	**35**	119	1300	960	**530**	390	220	120
26	Unrelated	BuCy	No	7.3	1	Full	Off	930	52	113	1300	1110	590	420	**80**	120
29	Unrelated	TBI	Ab	11.0	1	Full	Off	**478**	**18**	54	2700	2130	1110	890	140	460
30	Unrelated	TBI	Ab	10.0	1	44%	Off	1290	53	**11**	4000	3280	1560	1440	200	560
32	Haploidentical	FluMel	3	4.3	1	Full	Off	1287	193	**<4**	2600	1920	960	730	340	360
33	Haploidentical	FluMel	3	4.2	1	Full	Off	834	58	97	3100	2600	1640	650	**90**	370
HLH survivors																
34	Sibling	TBI	No	12.1	1	Full	Off	691	43	62	2800	2160	1090	840	340	360
36	Unrelated	BuCy	Ab	13.5	2	Full	Off	742	44	123	2300	1820	1080	600	140	370
37	Unrelated	BuCy	No	7.0	1	Full	Off	823	82	159	2300	1730	780	760	300	250
38	Unrelated	BuCy	No	6.0	1	Full	Off	1033	42	168	1700	1290	700	530	190	240
39	Unrelated	TBI	Ab	9.1	1	Full	Off	740	83	123	2200	1890	900	860	180	150
Others																
43	XLP	Unrelated	BuCy	No	5.5	1	Full	Off	851	142	142	2600	1950	880	810	160	520
44	XLP	Unrelated	FluMel	No	2.5	1	Full	Off	549	**40**	**<4**	1200	900	**520**	290	170	120
47	40L	Sibling	BuCy	No	9.6	1	Full	Off	1016	173	121	4500	3740	2340	1170	180	630
48	40L	Sibling	BuCy	No	4.0	1	Full	Off	1020	113	115	1500	1140	**500**	590	150	210
49	CHS	Sibling	BuCy	No	13.0	1	82%	Off	957	166	98	2900	2040	1290	620	200	590
50	LAD	Unrelated	FluMel	No	2.0	1	Full	Off	648	65	**<4**	4600	3400	1840	1240	180	1060
51	CGD	Unrelated	FluMel	No	2.6	1	Full	Off	436	**30**	47	2600	2030	1300	570	340	230
52	IPEX	Unrelated	FluMel	3	3.6	1	21%	Off	484	54	50	1700	1390	780	580	**70**	260

3 indicates CliniMACS $CD3^+$ depletion; 34, CliniMACS $CD34^+$ selection; 40L, CD40 ligand deficiency; Ab, complement/antibody; CGD, chronic granulomatous disease; FluMel, fludarabine + melphalan-based regimen; mel, melphalan + campath only; NA, not available; sibling, matched sibling donor; TBI, total body irradiation + cyclophosphamide-based regimen; unrelated, matched unrelated donor.

Values in bold type were below the normal range for current age. Lymphocyte subsets were quantified by multiplying the absolute lymphocyte counts to the percentage of cells labeled in each category by multicolor flow cytometry. Normal values for the lymphocyte subsets were determined by testing 57 healthy children age >12 months at our institution.

*Campath alone.

HLH

Of the 9 patients with familial HLH, 4 received an unrelated donor graft, 2 received a sibling donor graft, and 3 received a parental donor graft (Table 1). All 6 patients with HLA-matched donors received myeloablative conditioning. One sibling donor recipient developed grade III GVHD and veno-occlusive disease of the liver and died at day 38 of multiorgan failure. The 5 remaining patients survive, at a median of 9.1 years post-HCT (Table 2). One of the unrelated donor survivors underwent a second unrelated donor HCT 8 months after the first HCT owing to disease recurrence. All survivors demonstrate full donor chimerism and complete immune reconstitution, with documented vaccine response, normal Ig levels, and IVIG independence (Table 3). All survivors also have evidence of endocrinopathy, ranging from obesity alone to hypothyroidism, short stature, and ovarian failure (Table 2).

All 3 haploidentical donor recipients died. All received fludarabine-based conditioning (2 with busulfan, 1 with melphalan) and grafts rendered TCD by $CD34^+$ enrichment. All required DLI post-HCT for active infections, and 1 also underwent a second haploidentical donor HCT. However, all 3 recipients died with active infections.

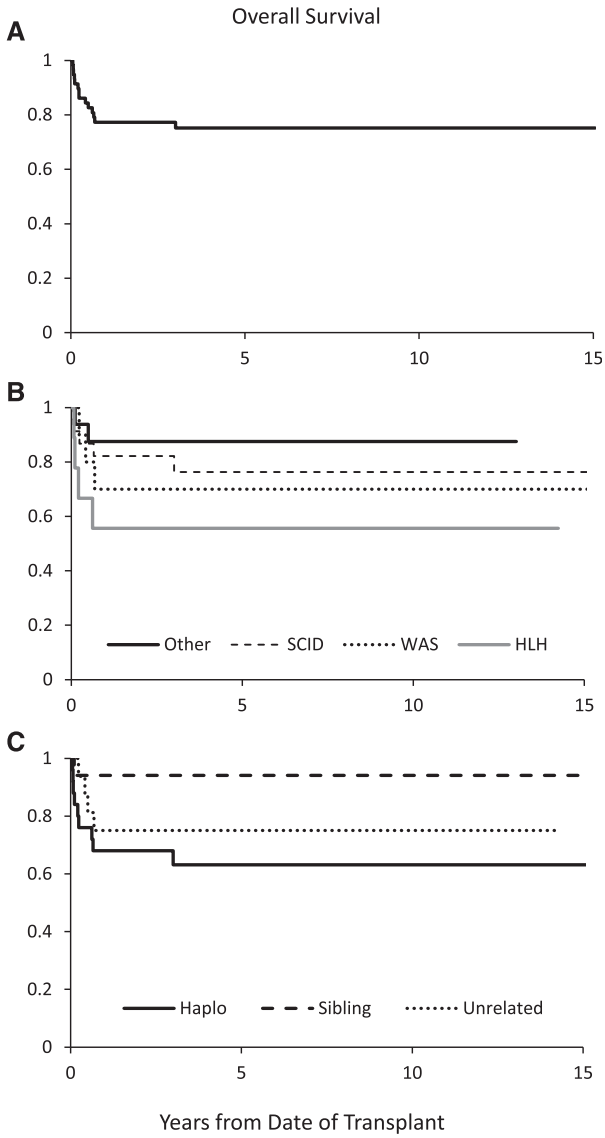

Figure 1. OS in all patients (A), by disease category ($P = .53$) (B), and by donor source ($P = .28$) (C).

Other Patients

The 10 remaining recipients included 4 with XLP, 2 with CD40L, 1 with IPEX, 1 with CHS, 1 with chronic granulomatous disease, and 1 with LAD. One patient with XLP who received TBI-based myeloablative conditioning and an unrelated donor graft died at day 182 post-HCT, and 1 patient with XLP who received BuCy-based myeloablative conditioning and a haploidentical donor graft died at day 41, both with invasive viral infections. Another patient with XLP currently survives 5.5 years after unrelated donor HCT with BuCy-based conditioning despite the development of grade IV acute GVHD. Both patients with CD40L and the patients with CHS have survived 4-13 years after sibling donor HCT with BuCy-based conditioning. The 4 remaining patients received fludarabine and melphalan RIC with unrelated donor grafts; all are clinically well, including the IPEX patient with mixed donor chimerism (Tables 2 and 3).

Group Analysis

Thirty-eight of 52 patients (73%) are currently alive, at a median of 7.7 years post-HCT. Fourteen patients died, at a median of 85 days after primary HCT (range, 20-1097 days). Invasive infection was a documented contributor in 12 of these 14 deaths (Table 2). Estimated OS was 75% ± 6% at 1 year post-HCT and 72% ± 8% at 6 years post-HCT (Figure 1A). Survival was not statistically different among patients with different diagnoses ($P = .53$) (Figure 1B). The 6-year OS was 91% ± 10% in sibling donor recipients, 75% ± 13% in unrelated donor recipients, and 63% ± 12% in parental donor recipients ($P = .28$) (Figure 1C).

Fifteen patients failed to achieve durable donor engraftment with their first HCT. Fourteen patients underwent a subsequent HCT, and 1 patient was managed with supportive care and enzyme replacement. EFS was used to estimate the probability of survival after achieving durable donor T cell engraftment with the first HCT. Patients who received a melphalan-based preparative regimen had a better EFS than those who received another preparative regimen ($P = .035$) (Figure 2A). The use of TCD grafts was associated with a worse EFS ($P = .008$) (Figure 2B); however, among the patients who received a TCD graft, EFS was significantly higher in those who received a CliniMACS $CD3^+$ TCD graft compared with those who received a graft rendered TCD by CliniMACS $CD34^+$ selection or complement/antibody methods ($P = .012$) (Figure 2C).

Only 8 patients (16%) developed grade II-IV acute GVHD after the first HCT, and 1 additional patient developed grade II GVHD after a second (with an unmanipulated parental graft) HCT. Despite the fact that the majority of TCD grafts were HLA-mismatched, only 3 of 32 (9%) TCD graft recipients developed grade II-IV acute GVHD. Conversely, 5 of 20 (25%) T cell–replete graft recipients developed grade II-IV acute GVHD after HCT. Of note, all 4 patients who developed grade III-IV GVHD received myeloablative conditioning, 3 of whom were unrelated donor transplant recipients. Five of the 9 patients who developed grade II-IV GVHD died, compared with 9 of 38 patients with grade 0 or I GVHD ($P = .1019$). Five patients developed chronic GVHD, of which 2 were extensive. Three patients had complete resolution, 1 patient remains on topical therapy only, and 1 patient remains on mycophenolate and topical therapy with limited skin involvement. None of the 11 patients who received a CliniMACS $CD3^+$ TCD graft developed grade II-IV acute GVHD or chronic GVHD.

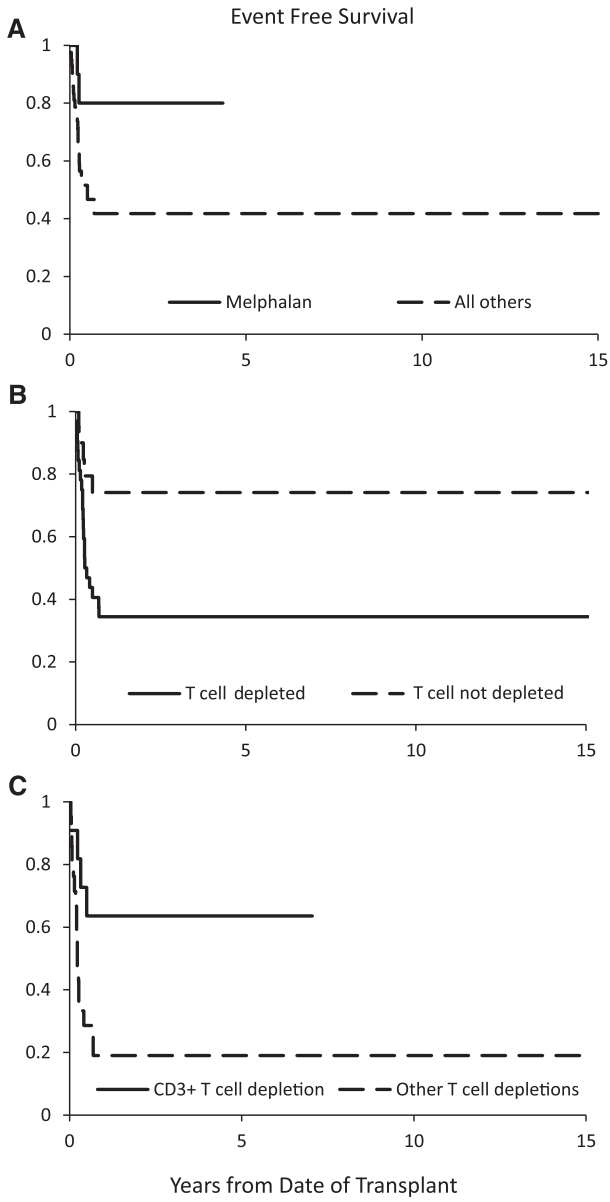

Figure 2. EFS by conditioning ($P = .035$) (A), TCD versus T cell replete ($P = .008$) (B), and by T cell depletion method ($P = .012$) (C).

Most survivors are clinically well and participate in normal daily activities (Table 2). However, medical problems related to the HCT procedure or to incomplete immune competency are frequent (Table 4). Eleven of 22 survivors who received a busulfan-based or TBI-based conditioning regimen are afflicted by endocrinopathies and other related toxicities. Six of 7 survivors who received no preparative chemotherapy for primary HCT are afflicted with chronic infections and require ongoing IVIG and antibiotic support. In all, only 7 of 29 survivors who did not receive a melphalan-based RIC are free of any current transplantation- or disease-related medical problems at last follow-up. Conversely, 8 of 9 survivors who received a melphalan-based RIC regimen are currently without medical problems (Tables 2 and 4).

Table 4. Patients with Transplantation-Related Morbidity at Last Follow-Up, by Conditioning Regimen

Complications	Bu/TBI	Melphelan	None
Survive without transplantation-related morbidity, n (%)	6 (19)	8 (80)	1 (9)
Survive with therapy-related morbidity, n (%)*	11 (35)	1 (10)	0
Endrocrine	9	0	0
Osteoporosis	2	1	0
Hypertension	2	0	0
Survive with immune-related morbidity, n (%)†	5 (16)	0	6 (55)
Chronic antibiotics	4	0	6
Chronic infection/warts	3	0	2
Deceased	9 (29)	1 (10)	4 (36)

*Therapy-related morbidity includes organ dysfunction associated with chemotherapy or radiotherapy given during the conditioning regimen, including endocrinopathy.

†Immune-related morbidity refers to complications from incomplete immunocompetency, such as chronic infections or the need for prophylactic antibiotics.

DISCUSSION

Allogeneic HCT remains the primary therapeutic option for the majority of patients with immune deficiency; however, the optimal transplantation approaches remain undefined [18]. Over the last 20 years, HCT for patients with immune deficiency at our institution has been largely successful, in that a majority of the patients survive and experience attenuation of their underlying disease regardless of disease type, donor source, or conditioning regimen. Mortality in this population is overwhelmingly related to invasive infections in the early posttransplantation period, emphasizing the need for rapid reconstitution of immune competency.

It is postulated that some degree of myeloid engraftment is required for long-term donor immune competency [8]. Our present findings provide strong support for the requirement of some level of pretransplantation myeloablation, even in patients with a significantly decreased immune barrier, such as those with SCID. However, full myeloablation was associated with significant therapy-related morbidity in survivors, given that both busulfan and TBI are associated with a number of therapy-related long-term morbidities, including endocrinopathies and second cancers [19-21]. It must be noted that one-half of our busulfan recipients received nontargeted oral dosing, which could have led to inappropriately high or low levels of exposure in those recipeints and thus may have predisposed them to worse outcome. Given this increased morbidity and the fact that RIC regimens are safe and effective in immunodeficient patients [22], the use of melphalan-based RIC has become institutional practice in recent years. The 10 patients who received melphalan-based RIC at our institution demonstrated an improved toxicity profile compared with their historical counterparts. Importantly, all survivors are currently free of the requirement for IVIG

and chronic antibiotics and are living normal lives. We postulate that this is related to full T cell engraftment and improved mixed myeloid and B cell chimerism [8], although lineage-specific chimerism was not routinely obtained in our patients. Certainly, patients who received melphalan-based RIC had a better EFS compared with the patients receiving other preparative regimens in our cohort ($P = .035$), indicating that sustained donor T cell engraftment was consistently obtained in this group. Other factors, however, such as improved supportive care and infection surveillance in recent years, might have contributed to the improved EFS.

Consistent with published literature, we found that when HLA-matched sibling donors are available, outcomes are particularly good regardless of underlying immune deficiency [5,6,14,23,24]. In addition, the sibling donor recipients have a uniformly good long-term clinical course. Unfortunately, a sibling donor was available for only a minority of recipients. The use of alternative donors, although effective in a majority of patients, is associated with worse outcomes compared with sibling donor transplants in immunodeficient recipients [5,6,14,23,24].

An HLA-matched unrelated donor is often considered when an appropriate sibling donor is not available. However, because many patients with immune deficiency present with illness and multiple active infections, they often are unable to wait the approximately 4 months [25] necessary to procure an unrelated donor graft (if available). Unrelated umbilical cord blood offers a rapidly available alternative donor source [26,27]; however, with these transplants, post-HCT donor lymphocytes are not available should the patient develop serious viral infection or a serious drop in donor chimerism.

Parental donors have the benefit of being rapidly available for virtually all patients, both before and after HCT. However, parental grafts typically require extensive T cell depletion, owing to the degree of HLA mismatch [28]. The rate of GVHD appeared to be lower in our TCD graft recipients, despite the fact that this group contained the preponderance of HLA-mismatched graft recipients. This indicates that T cell depletion is successful in controlling GVHD. However, the combination of T cell depletion and HLA mismatch begets delayed and often incomplete immune reconstitution, particularly if no conditioning is used [29]. As seen in malignant diseases [30], newer T cell depletion techniques, such as immunomagnetic $CD3^+$ cell depletion, seemed to improve donor engraftment and immune reconstitution in our parental donor recipients; their improved EFS indicated that they were more likely to survive and achieve successful donor engraftment without needing a subsequent HCT. Similar to other immunomagnetic T cell depletion techniques, $CD3^+$ cell depletion allows for an extensively TCD graft with a large hematopoietic stem cell dose; however, it also preserves important facilitator cells and innate immune cells, such as natural killer cells.

In conclusion, the majority of the immunodeficient patients who underwent HCT in our institution over the past 2 decades achieved cure of disease and currently participate in normal daily activities, regardless of their underlying disease and donor source. However, many survivors who received full myeloablation or no conditioning are burdened with either long-term toxicities or chronic infections. Melphalan-based RIC regimens were associated with less short-term and potentially less long-term toxicities in our patients. Immunomagnetic $CD3^+$ T cell depletion of mismatched grafts is effective in GVHD prevention, and also was associated with better EFS compared with complement/Ab-mediated depletion or $CD34^+$ enrichment. Therefore, the use of melphalan-based RIC regimens seems to safely provide sufficient myeloablation, which, together with efficient ex-vivo $CD3^+$ T cell depletion techniques, can safely achieve reliable donor immune reconstitution for patients with a variety of immune deficiencies. Future investigations of this transplantation platform in larger cohorts of patients are warranted to validate the findings reported herein and to further evaluate long-term donor immune competency.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2012.07.014.

REFERENCES

1. Gatti RA, Meuwissen HJ, Allen HD, et al. Immunological reconstitution of sex-linked lymphopenic immunological deficiency. *Lancet*. 1968;2:1366-1369.

2. Bach FH, Albertini RJ, Joo P, et al. Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome. *Lancet*. 1968;2:1364-1366.
3. Hacein-Bey-Abina S, Le Deist F, Carlier F, et al. Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. *N Engl J Med*. 2002;346:1185-1193.
4. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol*. 2004;22:625-655.
5. 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.
6. Buckley RH, Schiff SE, Schiff RI, et al. Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med*. 1999;340:508-516.
7. Patel NC, Chinen J, Rosenblatt HM, et al. Outcomes of patients with severe combined immunodeficiency treated with hematopoietic stem cell transplantation with and without preconditioning. *J Allergy Clin Immunol*. 2009;124:1062-1069.
8. Cavazzana-Calvo M, Carlier F, Le Deist F, et al. Long-term T-cell reconstitution after hematopoietic stem cell transplantation in primary T-cell–immunodeficient patients is associated with myeloid chimerism and possibly the primary disease phenotype. *Blood*. 2007;109:4575-4581.
9. Friedrich W, Honig M, Muller SM. Long-term follow-up in patients with severe combined immunodeficiency treated by bone marrow transplantation. *Immunol Res*. 2007;38:165-173.
10. Shizuru JA, Bhattacharya D, Cavazzana-Calvo M. The biology of allogeneic hematopoietic cell resistance. *Biol Blood Marrow Transplant*. 2010;16:S2-S7.
11. Dvorak CC, Cowan MJ. Hematopoietic stem cell transplantation for primary immunodeficiency disease. *Bone Marrow Transplant*. 2008;41:119-126.
12. Ozyurek E, Cowan MJ, Koerper MA, et al. Increasing mixed chimerism and the risk of graft loss in children undergoing allogeneic hematopoietic stem cell transplantation for nonmalignant disorders. *Bone Marrow Transplant*. 2008;42:83-91.
13. Rao K, Amrolia PJ, Jones A, et al. Improved survival after unrelated donor bone marrow transplantation in children with primary immunodeficiency using a reduced-intensity conditioning regimen. *Blood*. 2005;105:879-885.
14. Ozsahin H, Cavazzana-Calvo M, Notarangelo LD, et al. Long-term outcome following hematopoietic stem-cell transplantation in Wiskott-Aldrich syndrome: collaborative study of the European Society for Immunodeficiencies and European Group for Blood and Marrow Transplantation. *Blood*. 2008;111:439-445.
15. Howard V, Myers LA, Williams DA, et al. Stem cell transplants for patients with X-linked agammaglobulinemia. *Clin Immunol*. 2003;107:98-102.
16. Kaplan EL, Meier P. Nonparametric-estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
17. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep*. 1966;50:163-170.
18. Griffith LM, Cowan MJ, Kohn DB, et al. Allogeneic hematopoietic cell transplantation for primary immune deficiency diseases: current status and critical needs. *J Allergy Clin Immunol*. 2008;122:1087-1096.
19. Leung W, Ahn H, Rose SR, et al. A prospective cohort study of late sequelae of pediatric allogeneic hematopoietic stem cell transplantation. *Medicine (Baltimore)*. 2007;86:215-224.
20. Sanders JE. Growth and development after hematopoietic cell transplant in children. *Bone Marrow Transplant*. 2008;41:223-227.
21. Majhail NS, Brazauskas R, Rizzo JD, et al. Secondary solid cancers after allogeneic hematopoietic cell transplantation using busulfan-cyclophosphamide conditioning. *Blood*. 2011;117:316-322.
22. Amrolia P, Gaspar HB, Hassan A, et al. Nonmyeloablative stem cell transplantation for congenital immunodeficiencies. *Blood*. 2000;96:1239-1246.
23. Grunebaum E, Mazzolari E, Porta F, et al. Bone marrow transplantation for severe combined immune deficiency. *JAMA*. 2006;295:508-518.
24. Filipovich AH, Stone JV, Tomany SC, et al. Impact of donor type on outcome of bone marrow transplantation for Wiskott-Aldrich syndrome: collaborative study of the International Bone Marrow Transplant Registry and the National Marrow Donor Program. *Blood*. 2001;97:1598-1603.
25. Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med*. 1993;328:593-602.
26. Knutsen AP, Steffen M, Wassmer K, et al. Umbilical cord blood transplantation in Wiskott Aldrich syndrome. *J Pediatr*. 2003;142:519-523.
27. Knutsen AP, Wall DA. Umbilical cord blood transplantation in severe T-cell immunodeficiency disorders: two-year experience. *J Clin Immunol*. 2000;20:466-476.
28. Reisner Y, Kapoor N, Kirkpatrick D, et al. Transplantation for severe combined immunodeficiency with HLA-A, -B, -D, -DR incompatible parental marrow cells fractionated by soybean agglutinin and sheep red blood cells. *Blood*. 1983;61:341-348.
29. De Ravin SS, Malech HL. Partially corrected X-linked severe combined immunodeficiency: long-term problems and treatment options. *Immunol Res*. 2009;43:223-242.
30. Chen X, Hale GA, Barfield R, et al. Rapid immune reconstitution after a reduced-intensity conditioning regimen and a CD3-depleted haploidentical stem cell graft for paediatric refractory haematological malignancies. *Br J Haematol*. 2006;135:524-532.