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Clinical Research: Alternative Donors

Durable Chimerism and Long-Term Survival after Unrelated Umbilical Cord Blood Transplantation for Pediatric Hemophagocytic Lymphohistiocytosis: A Single-Center Experience

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A B S T R A C T

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disorder of immune dysregulation characterized by fever, hepatosplenomegaly, cytopenias, central nervous system disease, increased inflammatory markers, and hemophagocytosis. Currently, allogeneic hematopoietic stem cell transplantation is the only curative approach for patients with HLH, with reported survival ranging from 50% to 70% with myeloablative conditioning (MAC) regimens. However, donor availability and transplantation-related mortality associated with conventional MAC are major barriers to success. Unrelated umbilical cord blood transplantation (UCBT) provides a readily available alternative donor source for patients lacking matched related donors. Accordingly, we report the results of UCBT in 14 children treated between 1998 and 2016. All children received standard HLH chemotherapy before UCBT. The median age at diagnosis was 2.7 months (range, .8 to 10.4) and at transplantation was 7.5 months (range, 3.8 to 17). Ten patients received MAC with busulfan/cyclophosphamide/etoposide /antithymocyte globulin (ATG) (n = 5), busulfan/cyclophosphamide /ATG (n = 4), or busulfan /melphalan/ATG (n = 1). Four patients received reduced-toxicity conditioning (RTC) with alemtuzumab/fludarabine/melphalan/hydroxyurea ± thiotepa. Cord blood units were mismatched at either 1 (n = 9) or 2 (n = 5) loci and delivered a median total nucleated cell dose of 11.9×10^7/kg (range, 4.6 to 27.9) and $CD34^+$ dose of 3.1×10^5/kg (range, 1.1 to 6.8). The cumulative incidence of neutrophil engraftment by day 42 was 78.6% (95% confidence interval [CI], 42.9% to 93.4%) with a median of 19 days (range, 13 to 27), and that for platelet (50,000) engraftment by day 100 was 64.3% (95% CI, 28.2% to 85.7%) with a median of 51 days (range, 31 to 94). Six patients developed either grade II (n = 5) or grade IV (n = 1) acute graft-versus-host disease (GVHD); no extensive chronic GVHD was seen. Ten patients (71.4%) are alive and well at a median of 11.2 years after transplantation (range, .85 to 18.25), 9 of whom maintain sustained full donor chimerism after a single UCBT, whereas 1 patient with autologous recovery after first UCBT with RTC has achieved full donor chimerism after a second UCBT with MAC. This series demonstrates that, in combination with standard HLH therapy, UCBT after MAC or RTC conditioning can provide long-term survival with durable complete donor chimerism comparable to that of conventional donors. UCBT should be considered for patients with HLH lacking a fully matched related or unrelated adult donor.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a rare and often fatal syndrome of immune dysregulation and hyperactivation caused by defects in cytotoxicity of natural killer cells and cytotoxic T lymphocytes. Clinical manifestations include fever, hepatosplenomegaly, cytopenias, hemophagocytosis and, in some cases, neurologic manifestations [1-3]. Laboratory abnormalities include elevated ferritin, triglycerides, transaminases, bilirubin, soluble IL 2–receptor alpha-chain, and coagulopathy. Diagnosis is made by clinical presentation and fulfillment of 5 of 8 criteria as outlined in the HLH-2004 treatment protocol [4]. The disease

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can be genetically inherited or acquired after infections, malignancy, or autoimmune processes. Familial disease can be associated with 1 of several genetic defects (including mutations in PRF1, MUNC 13-4, STX11, STXBP2, XIAP, SH2D1A, etc.) with HLH as the sole manifestation. Additionally, HLH can be a part of immunodeficiency syndromes like Griscelli syndrome or Chediak-Higashi syndrome, where HLH is frequently, but not always, seen [5-8].

Although therapy with corticosteroids, etoposide, and cyclosporine is often effective at inducing disease remission and prolonging survival, the only cure for familial or primary HLH is allogeneic hematopoietic stem cell transplantation (HSCT) [4]. The procedure is also recommended in cases of HLH with central nervous system (CNS) involvement and in the setting of recurrent or progressive HLH despite first-line chemoimmunotherapy [9]. Although effective in curing a substantial number of patients, the conventional busulfan-based myeloablative preparative regimens have been associated with a very high rate of transplantation-related mortality (TRM) (~30% to 40%) and high incidence of acute liver and lung toxicities [10-14]. Reduced-intensity conditioning (RIC) using alternatives to busulfan, such as melphalan and treosulfan, have demonstrated superior overall survival [15,16]. However, a higher incidence of graft failure and mixed donor chimerism in these patients has necessitated frequent post-transplantation interventions, such as donor lymphocyte infusions and second transplantations [17,18].

Another challenge for these patients is finding a suitable donor quickly. Several patients lack conventional bone marrow donors, for whom partially mismatched unrelated umbilical cord blood transplantation (UCBT) can be an attractive alternative because of timely availability, need for less stringent HLA matching, and adequate cell dose in this population of young patients. There are limited data regarding long-term outcomes for pediatric HLH patients receiving UCBT [14,19-21]. In this report, we describe the outcomes after UCBT in children with HLH treated at a single center.

METHODS

Patients

Between 1998 and 2016, 14 pediatric patients lacking matched bone marrow donors underwent first single UCBT as definitive treatment for familial HLH at Duke University Medical Center. Data collection and retrospective analysis for this study were approved by the Duke University Medical Center institutional review board. Written assent or informed consent was obtained from all parents/caretakers, in accordance with the Declaration of Helsinki. Preliminary data on patients 11, 12, and 13 have been included in previous publications [22,23].

Donors

Cord blood units were selected based on HLA class I (A, B) intermediate-resolution and HLA class II (DRB1) high-resolution allelic level typing. The minimum requirement for precryopreserved total nucleated cell count was $> 3 \times 10^7$/kg and the cord blood donor had to be ≥ 4/6 HLA match with the recipient. Precryopreservation graft characteristics were obtained from the supplying cord blood banks. The Duke University Hospital stem cell laboratory thawed and performed testing of cord blood units providing the post-thaw data.

Conditioning Regimen

Ten patients received busulfan-based myeloablative conditioning (MAC). Five patients received busulfan 1 mg/kg every 6 hours for 16 doses from days –9 to –6; cyclophosphamide 50 mg/kg/dose from days –5 to –2 with mesna; etoposide 150 mg/m^2/dose from days –5 to –3, and equine antithymocyte globulin (eATG) 30 mg/kg/dose from days –3 to –1. Four patients received busulfan/cyclophosphamide/eATG without etoposide. One patient received busulfan, melphalan (45 mg/m$^2 \times 3$), and eATG as previous described [24]. Busulfan was administered by oral and intravenous route in 7 and 3 patients, respectively. Busulfan pharmacokinetics were studied after the first dose and doses 5 to 16 were adjusted to target steady state concentration of 600 ng/mL to 900 ng/mL. Four patients received reduced-toxicity conditioning (RTC) with alemtuzumab 1 mg/kg/dose i.v. on 3 successive days (–21 to –19), after an initial test dose of .2 mg/kg on day –22: hydroxyurea 30 mg/kg/day orally from day –22 to day –10; fludarabine 30 mg/m^2/day i.v. from days –9 to –5; melphalan 70 mg/m^2/day i.v. on days –4, –3. Three of these patient also received thiotepa 200 mg/m^2 i.v. on day –2. After thawing and washing, the cord blood units were infused intravenously on day 0, as previously described [25,26].

Graft-versus-Host Disease Prophylaxis and Treatment

Graft-versus-host disease (GVHD) prophylaxis for RTC transplantations started on day –3 with i.v. tacrolimus and i.v. mycophenolate 45 mg/kg/day. Tacrolimus levels were maintained between 8 ng/mL and 15 ng/mL. The remaining patients received cyclosporine and methylprednisolone 1 mg/kg/day (n = 9) or cyclosporine and mycophenolate (n = 1). Cyclosporine levels were maintained between 200 ng/mL and 300 ng/mL. For patients without evidence of GVHD, mycophenolate was discontinued on day 45, steroids were tapered after day 30, and calcineurin inhibitors were tapered starting at 9 months after transplantation. Acute and chronic GVHD were scored and graded in accordance with standard criteria [27,28]. Patients with grade 1 acute GVHD were treated with topical therapy while patients with grades 2 to 4 acute GVHD were treated with systemic methylprednisolone with or without additional agents.

Transplantation and Supportive Care

All patients were hospitalized in the pediatric blood and marrow transplant unit of Duke University Medical Center for conditioning and through engraftment and post-transplantation stabilization. All patients had central venous catheter access before transplantation. Levetiracetam or phenytoin was administered as seizure prophylaxis in patients while receiving busulfan. Baths were prescribed after thiotepa every 4 hours for 24 hours. Antiviral prophylaxis included acyclovir; antifungal prophylaxis included amphotericin B before 2003 (6 patients) and voriconazole thereafter (7 patients). Prophylaxis for veno-occlusive disease (VOD) was continuous low-dose heparin infusion (100 units/kg/day) from the day of initiating chemotherapy until day +28 for all patients except unique patient number (UPN) 14, who received ursodiol. Standard pneumocystis jiroveci pneumonia prophylaxis, nutritional, and transfusion support were administered as previously described [25].

All patients received intravenous immunoglobulin (500 mg/kg/dose) weekly until day + 100. Filgrastim (Amgen, Thousand Oaks, CA) was administered at 5 mcg/kg/day to 10 mcg/kg/day from day +1 and weaned after engraftment. Cytomegalovirus DNA was monitored weekly after transplantation. Two patients received irradiated granulocyte colony–stimulating factor–mobilized granulocytes from parental donors until engraftment, because of history of nonhealing deep perirectal ulcers and colostomy wound, respectively, at the time of transplantation.

Post-Transplantation Assessments

Donor cell chimerism was measured at engraftment, day 100, every 3 months during the first post-transplantation year, and yearly thereafter. Chimerism was confirmed by restriction fragment length polymorphism, microsatellite markers, HLA, or XY fluorescent in situ hybridization–based tests. Patients had organ function and immunologic evaluation every 3 months during the first year and annually thereafter. Patients also had serum ferritin and serum soluble IL-2 receptor levels measured wherever possible.

Statistical Analysis

We used primarily descriptive methods to evaluate the post-transplantation experience of this cohort. Continuous variables are described by the median and range, and nominal variables are summarized using frequencies and percentages. Overall survival (OS) was estimated using the Kaplan-Meier method. Follow-up time was calculated from the day of transplantation to the date of death or the date the patient was last known to be alive. Neutrophil and platelet engraftment were described by estimating the cumulative incidence function with death as a competing event. Analyses were performed in SAS 9.4 (SAS Institute, Cary, NC) and R 3.1.2.3.

RESULTS

Patient Characteristics

From 1998 to 2016, 14 patients with familial HLH underwent UCBT. All patients met the diagnostic criteria for HLH in accordance with the HLH-2004 protocol (Table 1) [4]. The median age of our cohort at the time of diagnosis of HLH was 2.7 months (range, .8 to 10.4). None of the patients had a family history of HLH. Mutation results were available for the last 6 patients, 4 of whom had PRF1 gene mutations

Table 1
HLH Criteria

HLH Diagnostic Criteria [4]	UPN													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fever	x	x	x	x	x	x	x		x	x	x	x	x	x
Splenomegaly	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Cytopenias ≥ 2 lineages	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hypofibrinogenemia (≤150 mg/dL) and/or hypertriglyceridemia (≥265 mg/dL)	x	x	x	x	x	x	x	x		x		x		x
Hemophagocytosis	x	x	x	x	x	x	x	x	x		x		x	
NK cell activity (reduced/absent)								x		x	x	x	x	
Serum ferritin (≥500 ng/mL)		x		x			x	x	x	x	x	x	x	x
sCD25/sIL2R (≥2400 U/mL)							x	x			x	x	x	x
Gene affected									MUNC	PRF1	MUNC	PRF1	PRF1	PRF1
Mutation									551 G > T 3173 T > C 3264 G > A	442 G > A 442 G > A	2346_2349 del GGAG	272 C > T	272 C > T	50delT 350_356 delinsATGC
Inheritance									CH	homo	Het	Het	Het	CH

NK indicates natural killer; CH, compound heterozygous; homo, homozygous; Het, heterozygous.

and 2 had MUNC mutations. Three of the 6 patients with documented mutations had heterozygous mutations, 2 patients had compound heterozygous mutations, and 1 patient had a homozygous mutation. Five patients (36%) had a pretransplantation history of respiratory insufficiency needing mechanical ventilation and 11 patients (79%) had a history of coagulopathy/disseminated intravascular coagulation. Six patients (43%) had evidence of CNS disease at the time of diagnosis. UPN 3 presented with seizures and had developed right hemiplegia before transplantation. UPN 4 developed disseminated *Klebsiella* and *Escherichia coli* infections associated with hepatitis, colitis, and bilateral rectal abscesses. She underwent diverting colostomy before transplantation, which was reanastomosed about 6 months post-transplantation. One patient (UPN 5) developed a clinical picture consistent with acute myeloid leukemia (AML) close to transplantation (blasts in peripheral blood, marrow, and spleen). UPN 12 had adenovirus infection in urine, stool, and blood before transplantation. UPN 13 had pseudomonas sepsis, acute respiratory distress syndrome, and deep perirectal ulcers before transplantation. Her hair features were typical of that seen in Griscelli syndrome, though molecular testing for RAB27A mutation was negative. Patients received HLH therapy per HLH-94 (n = 6) or HLH-2004 (n = 8) protocols. Four patients (29%) were determined to have active disease at the time of HSCT based on 1 or more of the following: bone marrow findings of hemophagocytosis or abnormal neurologic or cerebrospinal fluid findings. These included 3 patients with CNS disease noted during the immediate pretransplantation period. The median time to transplantation from start of standard HLH therapy was 115 days (range, 69 to 344). The median age at the time of transplantation was 7.5 months (range, 3.8 to 17.0). Median weight on day of transplantation was 8.3 kg (range, 4.2 to 12.9). Most patients were female (71%). All patients received 1 HSCT, except UNP 14. Patient and donor characteristics are summarized in Table 2.

Graft Characteristics

Using conventional HLA-matching criteria (low- or intermediate-resolution for -A and -B and high-resolution for -DRB1) to select grafts, donor-recipient matching was 5 of 6 in 9 patients (64%) and 4 of 6 in 5 patients (36%). The median precryopreserved total nucleated cell dose was 14.2×10^7 cells/kg (range, 6.75 to 37) and the median infused cell doses after thawing were 11.9×10^7 total nucleated cells/kg (range, 4.6 to 27.9), 3×10^5 $CD34^+$/kg (range, 1.1 to 6.8), and 11×10^4 total colony-forming units (CFU)/kg (range, .27 to 42.41). Donors matched for gender in 6 (43%) and ABO blood type in 9 (64%) recipients. Major and minor ABO mismatches were noted in 3 and 2 patient-donor pairs, respectively.

Neutrophil and Platelet Engraftment

The cumulative incidence of neutrophil engraftment by day 42 was 78.6% (95% confidence interval [CI], 42.9% to 93.4%) with a median of 19 days (range, 13 to 27). The cumulative incidence of platelet engraftment (50,000) by day 100 was 64.3% (95% CI, 28.2% to 85.7%) with a median of 51 days (range, 31 to 94).

Three patients experienced primary graft failure. One patient (UPN 14) who received RTC experienced autologous recovery on day 30. This patient underwent a second UCBT 8.6 months later with successful engraftment. The other 2 patients with primary graft failure received MAC (UPN 5 and 10). All 3 patients with graft failure had received cord blood units with low potency indicated by either low infused $CD34^+$ cell doses ($<1.5 \times 10^5$/kg) or poor growth of progenitors in the CFU assay ($.27 \times 10^4$/kg). There have been no secondary graft failures at most recent follow-up.

Donor Chimerism

All engrafted and surviving patients (9 of 14) demonstrated complete donor chimerism when last tested at a median of 10 years (range, 4.08 to 11.11) after transplantation and performed by HLA, fluorescent in situ hybridization XY technique, or short tandem repeat-based molecular method. In the patients tested more recently, chimerism was tested on $CD3^+$ and $CD15^+$ fractions and was found to be complete donor in both the fractions. One additional patient (UPN 14) achieved full donor chimerism after a second UCBT. There are no mixed chimeras in survivors in our study cohort.

GVHD

Six patients developed acute GVHD grades 2 to 4 (grade 2 [n = 5] and grade 4 [n = 1]). All patients with grade 2 GVHD were steroid responsive and did not require second-line agents against GVHD. UPN 6, who died on day 32, had evidence of liver GVHD on autopsy. No extensive chronic GVHD was seen in this cohort. All surviving patients are currently off immunosuppression with no evidence of GVHD by clinical exam or laboratory data.

Table 2
Characteristics and Outcomes

Pretransplantation Characteristics and Conditioning Regimen

UPN	Sex	Age at Dx, mo	Age at UCBT, mo	Pretransplantation Mechanical Ventilation	Pretransplantation Coagulopathy	CNS disease at Diagnosis	Therapy before UCBT	Preparative regimen	GVHD prophylaxis
1	F	.8	3.8	N	Y	N	HLH94	Bu/Cy/ATG	CSA/steroids
2	F	5.2	9.7	N	N	N	HLH94	Bu/Cy/ATG	CSA/steroids
3	M	7.4	10.4	Y	Y	Y	HLH94	Bu/Cy/ATG	CSA/steroids
4	F	2.3	4.8	N	Y	N	HLH94	Bu/Cy/ETOP/ATG	CSA/steroids
5	M	4.1	9.7	Y	Y	Y	HLH94	Bu/Mel/ATG	CSA/steroids
6	F	7.4	11.8	N	Y	Y	HLH94	Bu/Cy/ATG	CSA/steroids
7	F	3.6	6.3	N	Y	N	HLH2004	Bu/Cy/ETOP/ATG	CSA/steroids
8	F	1.1	4.6	N	Y	Y	HLH2004	Bu/Cy/ETOP/ATG	CSA/steroids
9	M	10.4	17.0	Y	Y	N	HLH2004	Bu/Cy/ETOP/ATG	CSA/steroids
10	M	1.7	13.0	N	Y	N	HLH2004	Bu/Cy/ETOP/ATG	CSA/MMF
11	F	1.9	5.4	N	N	Y	HLH2004	Campath/HU/Flu/Mel/TT	FK506/MMF
12	F	2.6	6.9	Y	Y	Y	HLH2004	Campath/HU/Flu/Mel/TT	FK506/MMF
13	F	2.0	5.3	Y	N	N	HLH2004	Campath/Flu/Mel	FK506/MMF
14	F	2.9	8.2	N	Y	N	HLH2004	Campath/HU/Flu/Mel/TT	FK506/MMF

Graft Characteristics and Transplantation Outcomes

No.	HLA Match	TNC $\times 10^7$/kg infused	CD34 $\times 10^5$/kg infused	CFU $\times 10^4$/kg infused	ANC > 500	Platelets > 50,000	aGVHD	cGVHD	Post-Transplantation Complications	Donor Chimerism at Last F/U	F/U (yr)
1	5	12.1	5.45	8.5	15	31	II skin	none	E. faecalis bacteremia	100	18.2
2	5	7.4	1.5	17.0	22	69	II skin	none	CMV viremia, cataracts	100	17.9
3	5	4.55	2.18	5.9	17	75	I skin	none	PRES, ARDS, staph aureus sepsis, C. diff colitis, acute renal insufficiency; residual neurologic deficits	100	17.3
4	4	16.1	6.44	15.3	14	94	II skin, liver	none	Fibular osteochondroma	100	16.6
5	5	11.5	1.38	8.6	Failure	NE		n/a	PGF, ARDS, VOD, MSOF	NE	D +34
6	5	11.2	4.7	6.7	21	NE	IV liver	n/a	VOD, Liver GVHD	99.5	D +32
7	5	12	2.1	11.4	13	56	II skin	none	None	100	11.3
8	5	18.1	5.4	12.7	27	NE		n/a	DAH, ARDS, PHT, CMV, candida, parainfluenza	99.5	D+72
9	4	14.2	6.8	18.5	26	51	II skin	none	CMV and C. diff colitis	100	10.9
10	4	5.65	1.1	6.8	Failure	n/a		n/a	PGF, persistent HLH, disseminated candidiasis	NE	D+40
11	5	15.1	3.6	26.7	19	33		Limited skin	None	100	6.5
12	4	11.8	2.5	10.6	20	50		Limited skin	Adenoviremia, enterococcus bacteremia	100	6.4
13	5	27.9	6.1	42.4	16	32	I skin	None	Acinetobacter/Klebsiella/ enterobacter BSI, Autoimmune thyroiditis	100	4.6
14	4	10.8	1.1	.27	Failure (AR)	NE		n/a	None	0	.7

DX indicates diagnosis; F, female; N, no; Y, yes; Bu, busulfan; Cy, cyclophosphamide; CSA, cyclosporine; M, male; ETOP, etoposide; MMF, mycophenolate mofetil; Mel, melphalan; HU, hydroxyurea; Flu, fludarabine; TT, thiotepa; TNC, total nucleated cell; ANC, absolute neutrophil count; aGVHD, acute graft-versus-host disease; cGHVD, chronic graft-versus-host disease; F/U, follow-up; CMV, cytomegalovirus; PRES, posterior reversible encephalopathy syndrome; ARDS, acute respiratory distress syndrome; C. diff, clostridium difficile; NE, not evaluated; PGF primary graft failure; MSOF, multisystem organ failure; DAH, diffuse alveolar hemorrhage; PHT, pulmonary hypertension; BSI, blood stream infection; AR, autologous recovery.

Immune Reconstitution

All surviving patients have been off immunosuppression and have had normal immune recovery with normal lymphocyte subsets, intravenous immunoglobulin independence, and normal response to both killed and live immunizations. No chronic infections or secondary malignancies have been reported during post-transplantation follow-up to date. UPN 14, who recently underwent a second UCBT, has resumed GVHD prophylaxis.

Survival

Ten of 14 patients are alive at the most recent follow-up with an OS of 71.4% (95% CI, 40.6% to 88.2%) (Figure 1) and event-free survival of 64.3% (95% CI, 34.3% to 83.3%) (Figure 2) after a single UCBT. One patient (UPN 14) underwent a second UCBT and is engrafted with donor cells without evidence of HLH. Thus, the disease-free survival (DFS) in this cohort is 71.4%. Median follow-up of surviving patients is 11.2 years after transplantation (range, .85 to 18.25). Four patients died, all within 100 days after transplantation (Table 3). UPN 5 had significant pretransplantation morbidity including active HLH/AML, acute respiratory distress syndrome, ascites, and prior splenectomy. He died on day 34 after transplantation with primary graft failure, VOD, and recurrence of acute respiratory distress syndrome. UPN 6 engrafted neutrophils on day 21 but subsequently developed VOD associated with increased busulfan exposure, rapidly progressed to fulminant hepato-renal syndrome, and died on day 32 after transplantation. Autopsy demonstrated evidence of both severe GVHD and VOD in the liver. UPN 8 engrafted on day 27; however, this patient developed severe pulmonary hypertension with diffuse pulmonary alveolar

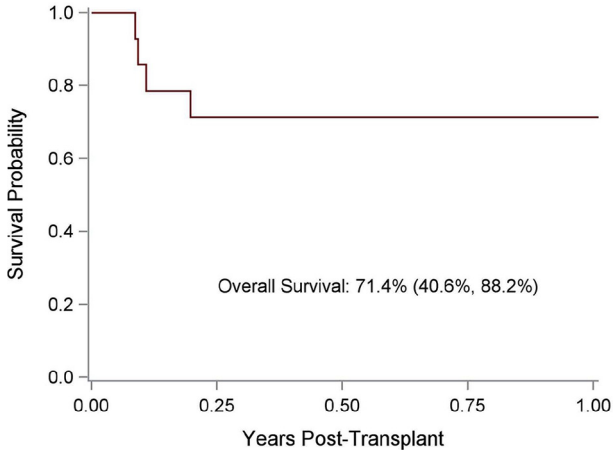

Figure 1. Overall survival.

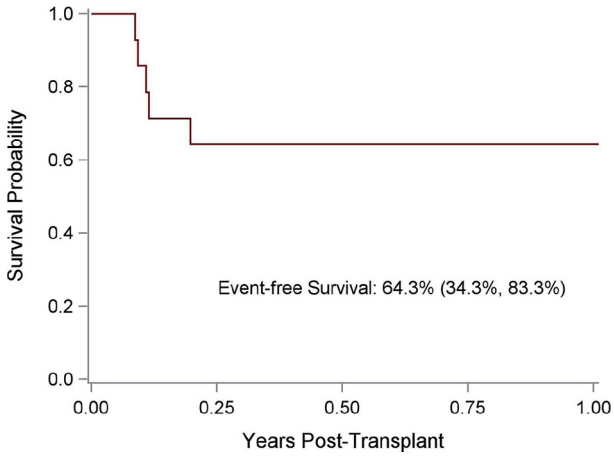

Figure 2. Event-free survival.

Table 3
Causes of Death

No.	Day after UCBT	Causes of Death
5	34	Primary graft failure, ARDS, recurrence VOD, MSOF
6	32	VOD, liver GVHD
8	72	DAH, ARDS, pulmonary HT, CMV, Candida parainfluenza
10	40	Primary graft failure, persistent HLH, disseminated candidiasis (parapsilosis)

hemorrhage in association with multiple infections (cytomegalovirus, parainfluenza type 2, and candida) and died on day 72 after transplantation. UPN 10 died on day 40 after transplantation from primary graft failure along with persistent HLH and disseminated candida parapsilosis infection.

DISCUSSION

We report long-term outcomes in a single-center cohort of 14 consecutive pediatric HLH patients undergoing unrelated UCBT with a median follow-up of > 11 years. OS, DFS, and event-free survival in our cohort (71.4%, 71.4%, and 64.2%) were similar to those reported in the literature involving

Table 4
Literature Review: UCBT and HLH

Study/First Author/Reference	Publication Year	n	Engraftment	OS
CIBMTR/Baker [10]	2008	9	100%	67%
AIEOP/Cesaro [11]	2008	6	-	83.3%
COBLT/Frangoul [19]	2010	5	60%	40%
Korean registry/Yoon [28]	2010	4	50%	25%
Japan/Ohga [27]	2010	28	-	71%
HLH94/Trottestam [14]	2011	10	-	80%
Japan/Nishi [17]	2012	13	69%	85%
Japan/Sawada [18]	2013	38	-	63%
Current study	2017	14	78.6%	71.4%

CIBMTR indicates Center for International Blood and Marrow Transplant Research; AIEOP, Italian Association of Pediatric Hematology and Oncology.

mainly bone marrow as graft source [10-13]. These data demonstrate the feasibility of cord blood as an alternative graft source in pediatric HLH patients undergoing allogeneic HSCT.

Most of the studies of allogeneic HSCT in pediatric HLH report use of matched bone marrow or peripheral blood as the graft source. Often, patients do not have suitably matched bone marrow donors. For these patients, partially mismatched unrelated umbilical cord blood is an attractive option for a variety of reasons, such as easy avaialbility, decreased incidence of GVHD, and adequate cell dose because of the young age of these patients. Data on unrelated UCBT in familial HLH are limited and are summarized in Table 4 [10,11,14,19-21,29,30]. Another alternative for patients lacking matched bone marrow donors is haploidentical transplantation from a related donor; however, the data are also limited so far. The largest series reported engraftment rates of 70% to 80% and survival of 50% [12,13]. However, recent advances in haploidentical transplantation have enabled an increasing use of this donor source and outcomes of haploidentical transplantations are likely to improve in the future.

One of the reported disadvantages of cord blood graft is a higher rate of engraftment failure. Graft failure rates as high as 30% to 50% have been reported in pediatric HLH patients undergoing UCBT [19,20,30]. We were able to demonstrate a high rate of engraftment in our cohort with a cumulative incidence of neutrophil engraftment of 78.6%. However, 3 of the 14 patients in our cohort failed to engraft, 2 of whom had received MAC and 1 received RTC regimen. The total nucleated cell dose received by these patients was adequate, though a negative impact of low cord blood potency demonstrated by either low $CD34^+$ cell dose (all received $< 1.5 \times 10^5$/kg) or low progenitor growth on CFU assay is possible [31]. One of the patients with graft failure had active disease evolving into AML and associated VOD, whereas the other patient was found to have persistent HLH and disseminated candida infection after transplantation. These factors could have further adversely influenced engraftment in these patients. The patient with autologous recovery after an RTC regimen was able to undergo a successful second UCBT with MAC.

TRM is a major cause of failure with use of MAC in this population of very young patients with significant preexisting comorbidities, with a high incidence of pulmonary, gut, and hepatic toxicities, including a high incidence of VOD. Myeloablative regimens are also associated with long-term sequelae, including infertility and risk of secondary malignancy, side effects that are particularly detrimental in the very young [32]. Consistent with the experience of larger studies,

all of the deaths in our cohort were noted in patients undergoing MAC regimens, mostly due to regimen-related toxicity (Table 3). There were no deaths in patients undergoing RIC, but there was 1 primary graft failure.

Because TRM is a major barrier to successful transplantation after MAC in HLH, approaches to decrease TRM are needed. In an earlier report from Histiocyte Society's HLH-94 study, mixed chimerism was noted in 8 of 43 evaluable patients, who were all disease free at a median of 3 years after transplantation, suggesting that mixed chimerism was sufficient to cure patients with HLH [13]. Similarly, other reports have suggested that ~20% to 30% donor chimerism could be the threshold for sustained disease control [12,33,34]. Since mixed chimerism can be an accepted outcome, attempts at decreasing the regimen intensity are logical. More recently, there have been several reports of successful use of RIC regimens in patients with HLH undergoing bone marrow transplantation, thus validating this concept [16-18,35]. Considering possible RIC approaches, alemtuzumab has been shown to be effective in salvage therapy of patients with refractory HLH, perhaps because of its profound lymphodepleting ability as well as its ability to target many of the immune cells, such as monocytes, macrophages, and dendritic cells, which are hyperactivated and dysregulated in patients with HLH [36]. Regimens combining alemtuzumab, melphalan, and fludarabine have been shown to improve survival in patients undergoing bone marrow transplantation for HLH. However, a high incidence of mixed donor chimerism has been associated with this approach [15-17].

There are very limited data on use of UCBT in patients with HLH and even less so with RIC (Table 4) [14,19,20]. Sawada et al., from a Japanese Registry with 38 patients, reported 62% OS and 59% DFS at 2 years [20]. Nishi et al. reported specifically on use of RIC (fludarabine/melphalan + low-dose total body irradiation) using cord blood in 13 patients with HLH. They observed an OS of 85%, though graft failure of nearly 30% was encountered [19]. Because of concerns for increased rejection in nonmalignant diseases with UCBT after alemtuzumab, fludarabine, and melphalan [37], we modified the regimen by adding hydroxyurea and thiotepa (3 of 4 patients), which has been noted to promote engraftment. We elected to avoid any radiation because of the age of these patients. This RTC regimen was very well tolerated in all 4 patients. Specifically, none of the patients developed VOD or liver or lung toxicity. One of the patients, who had lung toxicity before transplantation, was able to wean off oxygen and remains well with normal pulmonary function > 4 years after transplantation. Three of the 4 patients achieved sustained complete donor chimerism with this RTC regimen; the patient with autologous recovery after RTC regimen achieved full donor chimerism after a second UCBT with MAC regimen. Immune reconstitution has been excellent in all long-term engrafted patients, with all of them off intravenous immunoglobulin and having normal cellular immune phenotype and humoral immunity (IgG and IgA).

In conclusion, our experience suggests that unrelated cord blood is an effective graft source for pediatric patients with HLH lacking conventional donors. Because of prompt availability of UCB grafts, patients may avoid delays in transplantation with the potential of decreasing comorbidities and better outcomes. RTC regimens are an attractive alternative because of the significant pretransplantation comorbidities in these patients. UCBT for pediatric patients with HLH deserves further study in a larger prospective clinical trial.

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