Title

Unrelated Donor Transplantation in Children with Thalassemia using Reduced-Intensity Conditioning: The URTH Trial

Journal

Transplantation and Cellular Therapy, 24(6)

ISSN

2666-6375

Authors

Shenoy, Shalini
Walters, Mark C
Ngwube, Alex
et al.

Publication Date

2018-06-01

DOI

10.1016/j.bbmt.2018.01.023

Peer reviewed

HHS Public Access

Author manuscript

Biol Blood Marrow Transplant. Author manuscript; available in PMC 2019 June 01.

Published in final edited form as:

Biol Blood Marrow Transplant. 2018 June ; 24(6): 1216–1222. doi:10.1016/j.bbmt.2018.01.023.

Unrelated Donor Transplantation in Children with Thalassemia using Reduced Intensity Conditioning - The URTH Trial

Shalini Shenoy, MD[1], **Mark C. Walters, MD**[2], **Alex Ngwube, MD**[3], **Sandeep Soni, MD**[4,20], **David Jacobsohn, MD**[5], **Sonali Chaudhury, MD**[6], **Michael Grimley, MD**[7], **Kawah Chan, MD**[7], **Ann Haight, MD**[8], **Kimberley A. Kasow, DO**[9], **Suhag Parikh, MD**[10], **Martin Andreansky, MD, PhD**[11], **Jim Connelly, MD**[12], **David Delgado, MD**[13], **Kamar Godder, MD**[14], **Gregory Hale, MD**[15], **Michael Nieder, MD**[15], **Michael A. Pulsipher, MD**[16], **Felicia Trachtenberg, PhD**[17], **Ellis Neufeld, MD**[18], **Janet L. Kwiatkowski, MD**[19], and **Alexis A. Thompson, MD**[6]

[1]Washington University and St. Louis Children's Hospital, St. Louis, MO

[2]UCSF Benioff Children's Hospital, Oakland, CA

[3]Phoenix Children's Hospital, Phoenix, AZ

[4]Nationwide Children's Hospital, Columbus, OH

[5]Children's National Medical Center, Washington DC

[6]Ann and Robert H. Lurie Children's Hospital of Chicago and Northwestern University, Chicago, IL

[7]Texas Transplant Institute, San Antonio, TX

[8]Children's Healthcare of Atlanta and Emory University School of Medicine, Atlanta, GA

[9]University of North Carolina, Chapel Hill, NC

[10]Duke University Medical Center, Durham, NC

[11]University of Miami and Holtz Children's Hospital, Miami, FL

[12]University of Michigan, Ann Arbor, MI

[13]Riley Children's Hospital, Indianapolis, IN

[14]Nicklaus Children's Hospital, Miami, FL

[15]Johns Hopkins All Children's Hospital, St. Petersburg, FL

Correspondence: Shalini Shenoy, MD, Washington University School of Medicine, Box 8116, 660 S. Euclid Ave, St. Louis, MO, 63110, Phone: 314-454-6018, Fax: 314-454-2780, shalinishenoy@wustl.edu.

Author Contribution

S.S., M.C.W., A.N., S.So., M.A.P., and A.A.T. designed the trial, analyzed and interpreted the data, and drafted the manuscript; all remaining authors critically reviewed the manuscript; and all authors approved the final manuscript.

Conflict-of-interest disclosure:

The authors declare no competing financial interests.

[16]Children's Hospital Los Angeles, Los Angeles, CA

[17]New England Research Institutes

[18]St. Jude Children's Research Hospital

[19]Children's Hospital of Philadelphia, Perelman School of Medicine of the University of Pennsylvania

[20]Lucile Packard Children's Hospital, Stanford University, Palo Alto, CA

Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) can cure transfusion-dependent thalassemia (TDT). In a multicenter trial, we investigated the efficacy of reduced intensity conditioning (RIC) before unrelated donor (URD) HSCT in children with TDT. Thirty-three children (1–17 years), received marrow or cord blood (UCB) allografts. Median time to neutrophil engraftment was 13 (range, 10–25) and 24 (range, 18–49) days; platelet engraftment was 23 (range, 12–46) and 50 (range, 31–234) days after marrow and UCB respectively. With median follow-up of 58 (range, 7–79) months, overall and thalassemia-free survival was 82% (95% CI, 0.64–0.92) and 79% (95% CI, 0.6–0.9) respectively. The cumulative incidence (CI) of grade II–IV acute graft-versus-host disease (GVHD) after marrow and UCB was 24% and 44%; the 2-year CI of chronic extensive GVHD was 29% and 21% respectively; 71% (marrow) and 91% (UCB) recipients discontinued systemic immunosuppression by 2 years. Six patients who had Pesaro risk class 2 (N=5) and class 3 (N=1) died of GVHD (N=3), viral pneumonitis (N=2) and pulmonary hemorrhage (N=1). Outcomes following this RIC compared favorably with URD HSCT outcomes for TDT and supported engraftment in 32 of 33 patients. Efforts to reduce GVHD and infectious complications are being pursued further.

Keywords

thalassemia; reduced intensity conditioning; hematopoietic stem cell transplant; unrelated donor

Introduction

β-thalassemia is caused by mutations that reduce or abrogate β-globin synthesis. The accumulation of excess α-globin tetramers precipitate and trigger ineffective erythropoiesis.[1] Patients with homozygous or compound heterozygous hemoglobin subunit beta null mutations develop severe anemia early in life and require lifelong regular red blood cell (RBC) transfusions (transfusion dependent thalassemia, TDT) and iron chelation therapy to prevent organ toxicity from accumulated iron.[2] This cumbersome and expensive medical therapy and the associated risks of iron-overload and organ toxicity have stimulated investigations of allogeneic hematopoietic stem cell transplantation (HSCT) for this condition, and more recently, gene-modified infusions of autologous hematopoietic cells as curative alternatives.

Conventional myeloablative HSCT for TDT with a human leukocyte antigen (HLA)-matched sibling donor (MSD) has generated excellent results especially in young patients

with a low Pesaro risk score.[3,4] Alternative donor HSCT can establish donor erythropoiesis in those that lack MSD. However, thalassemia patients that are older, or with iron-related complications, and those undergoing unrelated donor (URD) myeloablative HSCT especially from cord blood, have a higher risk of toxicities, graft rejection, graft-versus-host disease (GVHD), and transplant related mortality (TRM) compared with MSD HSCT.[5,6]. Reduced intensity conditioning (RIC) might represent a strategy to decrease chemotherapy related organ toxicity of HSCT, especially if the risk of graft rejection can be mitigated by the immunosuppressive intensity of the regimen.

We describe the results of a prospective, multicenter Phase II clinical trial of unrelated donor HSCT in children with TDT that was conducted in collaboration with the Thalassemia Clinical Research Network (TCRN) and the Pediatric Blood and Marrow Transplant Consortium (PBMTC). The purpose of the trial was to test our hypothesis that an immunosuppressive RIC regimen was sufficient to establish donor hematopoiesis after URD bone marrow (BM) or umbilical cord blood (UCB) transplantation for TDT. The trial was referred to as the URTH (Unrelated Transplant for Thalassemia) trial.

Methods

Patients

Children with TDT, one to 16.99 years of age, who did not have suitable familial donors were eligible. TDT was established by genotype or was defined as requiring ≥ 8 erythrocyte transfusions per year of age. The first 20 patients were enrolled in NCT #01005576 and the next 13 were enrolled in an extension NCT #00920972 (stratum 2) after 2012. Patients were enrolled in sequence, first on the former, and next on the latter. Transplant methods and care were the same on both studies. Institutional Review Board approval for both studies was obtained at participating sites. All patients, and/or legal guardians assented/consented after detailed discussions about the potential benefits, risks and alternatives to participating in the trial had been conducted. Patient eligibility was not restricted by the Pesaro risk classification. A liver biopsy was required if RBC transfusions were administered for ≥1 year duration and serum ferritin level was ≥1000 ng/ml; patients with bridging fibrosis were excluded. Eligibility criteria also included adequate organ function, defined as left ventricular ejection fraction >40% or shortening fraction >26% by echocardiography, normal pulmonary function (normal diffusion capacity of the lung for carbon monoxide corrected for hemoglobin or oxygen saturation >97% on room air with normal chest radiograph in the very young), serum creatinine ≤ 1.5× the upper limit of normal for age or glomerular filtration rate >70 ml/min/1.73m^2, serum transaminases <5× upper limit of normal and a Lansky performance score of ≥70. Patients with uncontrolled infections, previous HSCT, or pregnant/lactating were excluded.

Stem cell sources and HLA typing

Patients were eligible to enroll if they had no HLA-matched family member and had a suitably matched unrelated marrow donor or cord product. URD BM donors were matched at HLA-A, -B, -C and –DRB1 loci by high resolution molecular typing. UCB products were matched at 5 or 6 loci (HLA-A, -B and -DRB1) following intermediate resolution typing at

class I and high resolution typing at the class II loci. In addition, a minimum pre-thaw total nucleated cell count (TNC) of ≥ 4×10^7/Kg recipient weight was required of the UCB product irrespective of red cell depletion. If suitable marrow and cord products were available, the choice of stem cell source was determined by the treating physician.

RIC regimen

Iron chelation was withdrawn 48 hours prior to commencing alemtuzumab. All patients received hydroxyurea (HU) (30 mg/kg/day oral) between days −50 and −22, alemtuzumab (3 mg test dose, then 10, 15, and 20 mg daily intravenously) between days −22 and −18, fludarabine (30 mg/m^2/day intravenously) between days −8 and −4, thiotepa (4 mg/kg × 2 intravenously) on day −4, and melphalan (140 mg/m^2 intravenously) on day −3. Donor hematopoietic stem cells were administered not less than 36 hours after the last dose of chemotherapy.

GVHD prophylaxis and classification

For GVHD prophylaxis, URD BM recipients received tacrolimus or cyclosporine between day -3 and +100 followed by a gradual taper to day +180 (in the absence of GVHD), methotrexate (7.5 mg/m^2 intravenously on days +1, +3 and +6) and methylprednisone/prednisone (1 mg/kg/day) between days +7 and +28. URD UCB recipients received tacrolimus or cyclosporine as above with mycophenolate mofetil (MMF) (15 mg/kg every 8 hours) between days +1 and +45. Acute GVHD (aGVHD) was graded from I to IV and chronic GVHD (cGVHD) was classified as limited or extensive according to Seattle criteria.[7,8]

Supportive care and infection prophylaxis

Filgrastim (5 μg/kg/d) was administered from day +7 until the absolute neutrophil count (ANC) was >1500/μL for three consecutive days. Herpes simplex prophylaxis with acyclovir, broad-spectrum anti-fungal and anti-bacterial agents were recommended for 6 months. Pneumocystis *jerovecii* prophylaxis was required for 1 year. Cytomegalovirus (CMV) surveillance of blood was mandated weekly until day +100 and subsequently with each clinic visit until day +180. Post-transplant iron chelation therapy could be resumed if indicated, after engraftment.

Outcomes

The primary end-point was thalassemia-free survival (TFS) at two years. TFS was defined as survival without graft rejection and resumption of chronic erythrocyte transfusions. Graft rejection was defined as <20% donor chimerism evaluated by DNA analysis in myeloid cells. Red cell chimerism was not evaluated. Overall survival (OS) was time from HSCT to last follow-up or death, and thus reflected transplant related mortality (TRM). Neutrophil recovery was defined as the first day of an ANC of ≥500/μL for three consecutive days; platelet engraftment was a platelet count of >20,000/μL independent of platelet transfusions for seven days. Engraftment was measured in peripheral blood enriched myeloid/lymphoid cells by the amplification of genes containing short tandem repeats. Immune reconstitution was evaluated as recovery of lymphocyte subsets (CD3, CD4, CD8, CD16/56, and CD19

cells) calculated by flow cytometry, and immunoglobulin levels at 6, 12 and 24 months post-HSCT. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria (CTC) for Adverse Events version 4.0. Sinusoidal Obstructive Syndrome (SOS) was defined according to Seattle criteria.[9] Hematogenous and invasive infections were reported. Patients were followed on study for a minimum of two years post-HSCT and encouraged to enroll after on the TCRN Longitudinal Cohort Study (NCT00661804) for long-term follow-up subsequently.

Statistical considerations

The study tested our hypothesis that this RIC regimen would be sufficient for donor cell engraftment and transfusion independence, with TFS of ≥75%. Stopping rules included graft rejection, treatment related mortality, or severe Grade IV aGVHD of >20%. TFS and OS were calculated using the Kaplan-Meier estimator and stratified by donor stem cell source. Continuous variables were summarized as median and range, and categorical variables as percentages. Pesaro risk class was determined for all patients but outcomes were not stratified by risk due to small numbers. The cumulative incidence method was used to estimate acute and chronic GVHD. In each case, death was a competing risk. Immune reconstitution was determined by flow cytometry analysis of lymphocyte subsets and recovery of serum immunoglobulin levels in the absence of infusion. Statistical analysis was performed using GraphPad Prism 6.0 (La Jolla, CA).

Results

Patient and donor characteristics

The study accrued patients from 14 centers nationwide. Donor and recipient characteristics are shown in Table 1. BM and UCB recipients were evenly distributed. β-globin genotype (when available), and dependency on regular RBC transfusions (in all patients) was confirmed prior to enrollment. While BM donors required 8 of 8 HLA-allele matched donor, 15 of 16 UCB recipients were matched at 5/6 HLA-loci. UCB recipients were younger than BM recipients (Table 1). The median ferritin level was 1214 (range, 421–3199) ng/ml. Nineteen patients underwent a pre-transplant liver biopsy; 7 were normal, 10 had mild fibrosis, two had moderate fibrosis, and none had bridging fibrosis. By the Pesaro risk classification, 12 children had class 1, 19 had class 2, and two had high-risk class 3 features. The cell dose infused is shown in Table 1. Sixty-one percent of patients (N=20) had an ABO mismatched donor. Twenty-four patients were CMV+ and at risk for reactivation. Of these, seventeen received transplants from a CMV seronegative donor (BM=4; UCB=13).

Engraftment and survival

The 2-year OS and TFS was 82.4% (95% CI 0.55–0.95) each for marrow and 81.3% (95% CI 0.54–0.95) and 75% (95% CI 0.47–0.91) respectively for UCB HSCT (Figure 1). Median follow up was 58 months (range, 7–79). The median time to neutrophil engraftment (ANC ≥500) was 13 days (range, 10–25) for marrow and 23.5 (range, 12–46) days for UCB (p=<0.0001). The median time to platelet engraftment was 24 (range, 18–49) days for marrow and 50.5 (range, 31–234) days for UCB (p=<0.0048) (Table II). No graft rejection was encountered in the BM group; one patient had graft rejection accompanied by

autologous recovery 39 days after UCB transplantation. The patient, a 5-year old, Pesaro risk class 2, was mismatched from the donor at the A antigen locus and had autologous recovery of neutrophils on day +35 and platelets on day +55. All other surviving patients had >95% donor chimerism in peripheral blood myeloid and lymphoid lineages at each time-point tested after HCST until 2 years that corresponded to normal hemoglobin levels and RBC transfusion independence.

GVHD, immunosuppression, and performance scores

The cumulative incidence of grade II-IV acute GVHD at day 100 was 24% and 44% in marrow and UCB recipients respectively (Table II). The skin and gut were the commonest organs involved; liver involvement was noted in two patients. Grade 4 GVHD occurred in three patients. The corresponding rates of chronic extensive GVHD at 2 years after marrow and UCB HSCT were 29% and 21% respectively. There was no significant association between GVHD incidence and the source of hematopoietic stem cells. However, 91% of UCB recipients weaned off systemic immunosuppression before 2 years post–HSCT compared with 71% of marrow recipients. The Lansky Performance Score at the most recent follow-up was 100% in all but 3 patients who reported scores of 90 (N=1) and 80 (N=2) (Table II).

Immune reconstitution

The tempo of immune reconstitution is shown in Figure 2. The panels include immune recovery status at 6, 12 and 24 months after HSCT in BM and UCB recipients. Immune recovery measures used were lymphocyte subset enumerations and immunoglobulin levels. When compared to the lower limit of normal (indicated as an interrupted horizontal line in each panel), T, B, and NK cell numbers and immunoglobulin levels returned to normal beginning at 6 months post-transplant. UCB recipients had lymphocyte recovery earlier than BM recipients. Cellular immune recovery correlated with infection patterns. Sixteen patients reported infectious complications (bacterial and viral) in the first 180 days post-HSCT. Infections documented beyond 180 days after HSCT included 7 patients who were receiving treatment for GVHD.

Toxicities

Two patients developed mild/moderate hepatic sinusoidal obstruction syndrome (SOS) after UCBT on days +24 and +29. Their ferritin levels were 1214 and 1630 ng/ml respectively; the latter had mild hepatic fibrosis. Both resolved uneventfully after supportive care alone (N=1) or with defibrotide (N=1). CMV reactivation in 13 of 16 UCB and 9 of 17 BM recipients included those at risk (CMV+ before HSCT) and was treated pre-emptively with systemic anti-viral therapy. Adenovirus (N=9) and EBV (N=9) viremia were also detected in the blood. One patient died of CMV pneumonitis and another died of adenovirus pneumonitis. All but one patient with EBV activation recovered without specific therapy. The exception was a patient with GVHD who developed post-transplant lymphoproliferative disorder (PTLD) with brain involvement that was fatal. Three patients died of GVHD related complications. Each had infectious complications during GVHD therapy. One patient died of diffuse alveolar hemorrhage. Pesaro risk scores in the six patients who died were Class 1

in one patient, Class 2 in 4 patients and Class 3 in one patient. Four deaths were early (<100 days after HSCT) and two were late (>1 year).

Discussion

Supportive care for thalassemia major includes lifelong RBC transfusions and iron chelation therapy. While survival among patients who strictly adhere to medical management is outstanding, it is associated with high-cost, is cumbersome, and impairs quality of life.[10] In those who do not or cannot adhere to supportive care, there is a shortened lifespan. Allogeneic HSCT is an attractive alternative to supportive care, but it is limited by the availability of HLA-matched MSD.[11] Hence, only a small fraction of patients receive this potentially curative therapy. Outcomes after HSCT have varied with patient age, stem cell source, and risk classification before HSCT.[6,12–14] The current TFS after conventional myeloablative HLA-matched sibling donor HSCT is 86% and 80% after marrow and UCB HSCT respectively.[4,11,15,16] Patients >7 years of age, with hepatomegaly, and those with Pesaro risk class 3 disease experience worse outcomes.[12,13] TFS after URD HSCT is 80% if Pesaro risk class 1 and 2, but 55% for risk class 3.[17] In addition to the risk of transplant-related mortality that increases in high-risk patients, HSCT outcomes are influenced by increased graft rejection that varies from 10 to 35%.[18] Rejection risks are presumably related to immunologic barriers to donor engraftment in these chronically transfused patients and are amplified in the presence of HLA disparity at low-expression loci such as HLA-DPB1.[5,19] Safer methods of transplant conditioning and myeloablation have generated better outcomes more recently. A TFS of 78% after MUD HSCT using treosulfan based myeloablative conditioning, 82% after pre-transplant courses of fludarabine and dexamethasone followed by standard busulfan and cyclophosphamide, or 90% after a myeloablative combination of busulfan, fludarabine, cyclophosphamide and thiotepa are examples of modifications in preparative regimens that have improved outcomes.[5,20,21] Outcomes after unrelated UCBT have been unsatisfactory. Registry data revealed a TFS of 21% after UCB HSCT from unrelated donors; overall, the graft rejection rate for hemoglobinopathy was 52%.[6] Graft rejection accompanied by marrow aplasia and other toxicities also caused fatal outcomes. Better outcomes (74% TFS) following unrelated UCBT for thalassemia were described in a single institution trial with myeloablative conditioning.[22]

The purpose of this trial was to evaluate URD HSCT, including marrow and UCB as a donor source, after RIC in children with TDT. The regimen met RIC designation based on recovery of autologous hematopoiesis within 28 days in the event of graft rejection as previously defined.[23] We reasoned that a RIC regimen in lieu of myeloablation might avoid a prolonged period of marrow aplasia if graft rejection occurred, and could reduce the risk of organ toxicity such as hepatic SOS and late gonadal failure.[24,25] A very similar RIC regimen generated excellent results after MSD HSCT and UCB transplants in children with TDT and sickle cell disease.[26–28] In addition, utilizing an URD in this study, and including marrow and UCB as stem cell source, mitigated the restriction of not having a MSD. The TFS observed in this study was similar to outcomes after myeloablative conditioning and URD HSCT in other studies of marrow or mobilized peripheral blood, and better than in a retrospective analysis of unrelated UCBT. Notably, the graft rejection rate was 3% after

UCBT in our series. Thus, in addition to achieving engraftment with marrow stem cells, we observed very good results after UCBT with little graft failure, representing an important advance toward using alternate stem cell sources in hemoglobinopathy transplants, especially since we have shown similar success in a pilot trial of unrelated UCB HSCT for sickle cell disease.[27]

All engrafted patients had full donor chimerism presumably attributable to the intensity of recipient immunosuppression provided (Table II). While outcomes were similar after UCB and marrow transplantation in the study population, neutrophil and platelet engraftment were significantly delayed following UCB compared to marrow. Even though 12 patients had portal fibrosis before HSCT, hepatotoxicity and lung toxicity occurred infrequently after HSCT, even in risk class 2 and 3 patients. In contrast to sickle cell disease, no one developed posterior reversible encephalopathy syndrome after HSCT even though it has been observed after HSCT for TDT suggesting a predisposition to this complication.[29,30] Infectious complications occurred frequently, and for the most part were viral in origin occurring early post-HSCT before immune reconstitution. CMV reactivation occurred in all high-risk patients after UCB HSCT. Viral (CMV and adenovirus) pneumonitis was fatal in 2 patients, after UCB and marrow transplantation respectively. Bacterial infections were self-limited and responded to treatment; no patient had invasive fungal infections. The tempo of immune reconstitution was rapid after the initial 6-month post-transplant period which was reflected in low infection rates thereafter. The rapid quantitative recovery of lymphocyte subsets following UCBT might indicate the lower incidence and severity of chronic GVHD in this group allowing faster immunosuppression taper in comparison to marrow recipients (Figure 2).

GVHD has no benefit after HSCT for non-malignant disorders but has a higher incidence after URD HSCT. The incidence of GVHD after marrow and UCB transplantation in this study was similar to the incidence previously described following myeloablative HSCT.[31] Though GVHD resolved earlier in UCB recipients allowing cessation of immunosuppressive agents earlier compared to marrow recipients, it was still an important cause of mortality. GVHD may have been influenced by the timing of administration of alemtuzumab. Alemtuzumab in a more ‘proximal’ time-frame before HSCT reduces the GVHD risk compared to ‘distal’ administration. However, the this benefit of proximal alemtuzumab is balanced by a higher risk of graft rejection or lower levels of donor chimerism compared with distal administration.[32] Despite the GVHD, performance scores at 1-year and longer following HSCT were high (Table II). Quality of life analyses are in progress on patients that consented to the same or enrolled on the TLC trial.

Our study demonstrates for the first time that durable engraftment can be achieved after URD marrow and UCB transplantation using a RIC regimen in children with TDT. Because we observed a high rate of graft rejection in SCD recipients who received a similar RIC before URD UCBT, we modified the RIC regimen to include hydroxyurea and thiotepa.[33–35] We have observed no predisposition to SOS or major late effects except hypothyroidism after HSCT with a similar regimen, and fertility was preserved in females that were of eligible age for evaluation.[36] However, long-term follow up is necessary after this RIC transplant to determine if late gonadal function is preserved especially in the vulnerable

iron-laden TDT patients.[37] Early infection risks, especially of viral origin, can be prevented and treated now more effectively with newer methods of pharmacologic prophylaxis and cellular therapy and have been adopted in currently ongoing trials.[38,39] In vitro UCB expansion methods allowing faster engraftment may further reduce the risk of post-transplant infections.[40,41] This regimen was sufficient for donor engraftment but GVHD remained a problem in susceptible patients similar to a trend described in sickle cell disease.[42,43] Graft manipulation such as T-cell depletion can reduce GVHD risk after HSCT from URD BM but is not feasible with UCB.[44] Better HLA matched UCB products can improve outcomes but this will further restrict donor availability.[45] In ongoing follow-up clinical trials, we have employed newer methods of GVHD prophylaxis such as abatacept following unrelated donor and post-transplant cyclophosphamide following haploidentical donor HSCT.[46,47] An ability to prevent late toxicities, infection risks, and GVHD will advance allogeneic HSCT and make it an attractive alternative to supportive care in the treatment of TDT. Efforts that expand the donor pool as in this report, or utilize autologous gene modification techniques that are underway, show promise in expanding curative options for this disorder.

Acknowledgments

This work was supported by grants from the National Institutes of Health U01 HL065238 [to New England Research Institutes/Thalassemia Clinical Research Network], U01HL069254 and a grant from the St. Baldrick's Foundation [to the Pediatric Blood and Marrow Transplant Consortium], and Children's Discovery Institute, Washington University in St. Louis [to Shenoy]. We gratefully acknowledge data supervision and audit support from Lisa Murray, Clinical Research Associate at Washington University in St. Louis, the Data Safety Monitoring Board of the Pediatric Blood and Marrow Transplant Consortium, and the New England Research Institutes for logistical support for the trial.

References

1. Thein S. Pathophysiology of beta thalassemia--a guide to molecular therapies. Hematology Am Soc Hematol Educ Program. 2005:31–7. [PubMed: 16304356]
2. Tubman V, Fung E, Vogiatzi M, et al. Guidelines for the Standard Monitoring of Patients With Thalassemia: Report of the Thalassemia Longitudinal Cohort. J Pediatr Hematol Oncol. 2015; 37:e162–9. [PubMed: 26201037]
3. Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. N Eng J Med. 1990; 322:417–421.
4. Locatelli F, Kabbara N, Ruggeri A, et al. Outcome of patients with hemoglobinopathies given either cord blood or bone marrow transplantation from an HLA-identical sibling. Blood. 2013; 122:1072–8. [PubMed: 23692854]
5. Locatelli F, Merli P, Strocchio L. Transplantation for thalassemia major: alternative donors. Curr Opin Hematol. 2016; 23:515–23. [PubMed: 27537474]
6. Ruggeri A, Eapen M, Scaravadou A, et al. Umbilical cord blood transplantation for children with thalassemia and sickle cell disease. Biol Blood Marrow Transplant. 2011; 17:1375–82. [PubMed: 21277376]
7. Shulman H, Sullivan K, Weiden P, et al. Chronic graft-versus-host syndrome in man: a long term clinicopathologic study of 20 Seattle patients. Am J Med. 1980; 69:204–17. [PubMed: 6996481]
8. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995; 15:825–8. [PubMed: 7581076]
9. McDonald G, Sharma P, Matthews D, et al. The clinical course of 53 patients with veno-occlusive disease of the liver after marrow transplantation. Transplantation. 1985; 39:603–8. [PubMed: 3890288]
10. Politis C. The psychosocial impact of chronic illness. Ann N Y Acad Sci. 1998; 30:349–54.

11. Baronciani D, Angelucci E, Potschger U, et al. Hemopoietic stem cell transplantation in thalassemia: a report from the European Society for Blood and Bone Marrow Transplantation Hemoglobinopathy Registry, 2000–2010. Bone Marrow Transplant. 2016; 51:536–41. [PubMed: 26752139]
12. Sabloff M, Chandy M, Wang Z, et al. HLA-matched sibling bone marrow transplantation for β-thalassemia major. Blood. 2011; 117:1745–50. [PubMed: 21119108]
13. Mathews V, George B, Deotare U, et al. A new stratification strategy that identifies a subset of class III patients with an adverse prognosis among children with beta thalassemia major undergoing a matched related allogeneic stem cell transplantation. Biol Blood Marrow Transplant. 2007; 13:889–94. [PubMed: 17640592]
14. Gaziev D, Galimberti M, Lucarelli G, et al. Bone marrow transplantation from alternative donors for thalassemia: HLA-phenotypically identical relative and HLA-nonidentical sibling or parent transplants. Bone Marrow Transplant. 2000; 25:815–21. [PubMed: 10808201]
15. Walters M, Quirolo L, Trachtenberg E, et al. Sibling donor cord blood transplantation for thalassemia major: Experience of the Sibling Donor Cord Blood Program. Ann N Y Acad Sci. 2005; 1054:206–13. [PubMed: 16339667]
16. Soni S, Boulad F, Cowan M, et al. Combined umbilical cord blood and bone marrow from HLA-identical sibling donors for hematopoietic stem cell transplantation in children with hemoglobinopathies. Pediatr Blood Cancer. 2014; 61:1690–4. [PubMed: 24803091]
17. La Nasa GAF, Giardini C, Pession A, Fagioli F, Caocci G, Vacca A, De Stefano P, Piras E, Ledda A, Piroddi A, Littera R, Nesci S, Locatelli F. Unrelated bone marrow transplantation for beta-thalassemia patients: The experience of the Italian Bone Marrow Transplant Group. Ann N Y Acad Sci. 2005; 1054:186–95. [PubMed: 16339665]
18. Galambrun C, Pondarré C, Bertrand Y, et al. French multicenter 22-year experience in stem cell transplantation for beta-thalassemia major: lessons and future directions. Biol Blood Marrow Transplant. 2013; 19:62–8. [PubMed: 22892550]
19. Fleischhauer K, Locatelli F, Zecca M, et al. Graft rejection after unrelated donor hematopoietic stem cell transplantation for thalassemia is associated with nonpermissive HLA-DPB1 disparity in host-versus-graft direction. Blood. 2006; 107:2984–92. [PubMed: 16317094]
20. Li C, Wu X, Feng X, et al. A novel conditioning regimen improves outcomes in β-thalassemia major patients using unrelated donor peripheral blood stem cell transplantation. Blood. 2012
21. Anurathapan U, Pakakasama S, Mekjaruskul P, et al. Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. Biol Blood Marrow Transplant. 2014; 20:2066–71. [PubMed: 25064743]
22. Jaing T, Hung I, Yang C, et al. Unrelated cord blood transplantation for thalassaemia: a single-institution experience of 35 patients. Bone Marrow Transplant. 2012; 47:33–9. [PubMed: 21383683]
23. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the center for international blood and marrow transplant research. Biol Blood Marrow Transplant. 2009; 15:367–9. [PubMed: 19203728]
24. De Sanctis V, Eleftheriou A, Malaventura C, et al. Prevalence of endocrine complications and short stature in patients with thalassaemia major: a multicenter study by the Thalassaemia International Federation (TIF). Pediatr Endocrinol Rev. 2004; 2(Suppl 2):249–55. [PubMed: 16462705]
25. Li C, Chik K, Won G, et al. Growth and endocrine function following bone marrow transplantation for thalassemia major. Pediatr Hematol Oncol. 2004; 21:411–9. [PubMed: 15205084]
26. King A, Kamani N, Bunin N, et al. Successful matched sibling donor marrow transplantation following reduced intensity conditioning in children with hemoglobinopathies. Am J Hematol. 2015; 90:1093–8. [PubMed: 26348869]
27. Abraham A, Cluster A, Jacobsohn D, et al. Reduced Intensity Unrelated Umbilical Cord Blood Transplantation for Sickle Cell Disease: Results of a Phase I Trial. Biol Blood Marrow Transplant. 2017

28. Soni S, Breslin N, Cheerva A. Successful unrelated umbilical cord blood transplantation for class 3 β-thalassemia major using a reduced-toxicity regimen. Pediatr Transplant. 2014; 18:E41–3. [PubMed: 24330091]
29. Gaziev J, Marziali S, Paciaroni K, et al. Posterior Reversible Encephalopathy Syndrome after Hematopoietic Cell Transplantation in Children with Hemoglobinopathies. Biol Blood Marrow Transplant. 2017
30. Noè A, Cappelli B, Biffi A, et al. High incidence of severe cyclosporine neurotoxicity in children affected by haemoglobinopaties undergoing myeloablative haematopoietic stem cell transplantation: early diagnosis and prompt intervention ameliorates neurological outcome. Ital J Pediatr. 2010; 36:14. [PubMed: 20181110]
31. La Nasa G, Vacca A, Littera R, et al. What Unrelated Hematopoietic Stem Cell Transplantation in Thalassemia Taught us about Transplant Immunogenetics. Mediterr J Hematol Infect Dis. 2016; 8:e2016048. [PubMed: 27872728]
32. Marsh R, Rao M, Gefen A, et al. Experience with Alemtuzumab, Fludarabine, and Melphalan Reduced-Intensity Conditioning Hematopoietic Cell Transplantation in Patients with Nonmalignant Diseases Reveals Good Outcomes and That the Risk of Mixed Chimerism Depends on Underlying Disease, Stem Cell Source, and Alemtuzumab Regimen. Biol Blood Marrow Transplant. 2015; 21:1460–70. [PubMed: 25865646]
33. Kamani N, Walters M, Carter S, et al. Unrelated donor cord blood transplantation for children with severe sickle cell disease: results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). Biol Blood Marrow Transplant. 2012; 18:1265–72. [PubMed: 22343376]
34. Gaziev J, Marziali M, Isgrò A, et al. Bone marrow transplantation for thalassemia from alternative related donors: improved outcomes with a new approach. Blood. 2013; 122:2751–6. [PubMed: 23963044]
35. Parikh S, Mendizabal A, Benjamin C, et al. Reduced-Intensity Conditioning (RIC) in children with nonmalignant disorders (NMD) undergoing unrelated donor umbilical cord blood transplantation (UCBT). Biol Blood Marrow Transplant. 2014; 20:326–36. [PubMed: 24296492]
36. Madden L, Hayashi R, Chan K, et al. Long-Term Follow-Up after Reduced-Intensity Conditioning and Stem Cell Transplantation for Childhood Nonmalignant Disorders. Biol Blood Marrow Transplant. 2016; 22:1467–72. [PubMed: 27164064]
37. Poomthavorn P, Chawalitdamrong P, Hongeng S, et al. Gonadal function of beta-thalassemics following stem cell transplantation conditioned with myeloablative and reduced intensity regimens. J Pediatr Endocrinol Metab. 2013; 26:925–32. [PubMed: 23729599]
38. Chemaly R, Shah D, Boeckh M. Management of respiratory viral infections in hematopoietic cell transplant recipients and patients with hematologic malignancies. Clin Infect Dis. 2014; 15:S344–51.
39. Saglio F, Hanley P, Bollard C. The time is now: moving toward virus-specific T cells after allogeneic hematopoietic stem cell transplantation as the standard of care. Cytotherapy. 2014; 16:149–59. [PubMed: 24438896]
40. Anand S, Thomas S, Hyslop T, et al. Transplantation of Ex Vivo Expanded Umbilical Cord Blood (NiCord) Decreases Early Infection and Hospitalization. Biol Blood Marrow Transplant. 2017; 23:1151–1157. [PubMed: 28392378]
41. Kiernan J, Damien P, Monaghan M, et al. Clinical Studies of Ex Vivo Expansion to Accelerate Engraftment After Umbilical Cord Blood Transplantation: A Systematic Review. Transfus Med Rev. 2017; 31:173–182. [PubMed: 28087163]
42. Gluckman E, Cappelli B, Bernaudin F, et al. Sickle cell disease: an international survey of results of HLA-identical sibling hematopoietic stem cell transplantation. Blood. 2016
43. Shenoy S, Eapen M, Panepinto J, et al. A BMT CTN phase II trial of unrelated donor marrow transplantation for children with severe sickle cell disease. Blood. 2016
44. Bertaina AMP, Rutella S, Pagliara D, Bernardo ME, Masetti R, Pende D, Falco M, Handgretinger R, Moretta F, Lucarelli B, Brescia LP, Li Pira G, Testi M, Cancrini C, Kabbara N, Carsetti R, Finocchi A, Moretta A, Moretta L, Locatelli F. HLA-haploidentical stem cell transplantation after

removal of αβ+ T and B cells in children with nonmalignant disorders. Blood. 2014; 124:822–6. [PubMed: 24869942]

45. Eapen M, Wang T, Veys P, et al. Allele-level HLA matching for umbilical cord blood transplantation for non-malignant diseases in children: a retrospective analysis. Lancet Haematol. 2017; 4:e325–e333. [PubMed: 28623181]
46. Koura D, Horan J, Langston A, et al. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: a first-in-disease trial. Biol Blood Marrow Transplant. 2013; 19:1638–49. [PubMed: 24047754]
47. Bolaños-Meade J, Fuchs E, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. Blood. 2012; 120:4285–91. [PubMed: 22955919]

Key points

- Hematopoietic stem cell transplantation from unrelated marrow and cord blood after reduced intensity conditioning resulted in high rates of engraftment in children with transfusion dependent thalassemia, in a prospective multicenter trial.
- Strategies that provide more effective prophylaxis against graft-versus-host disease and viral infections can improve outcomes further.

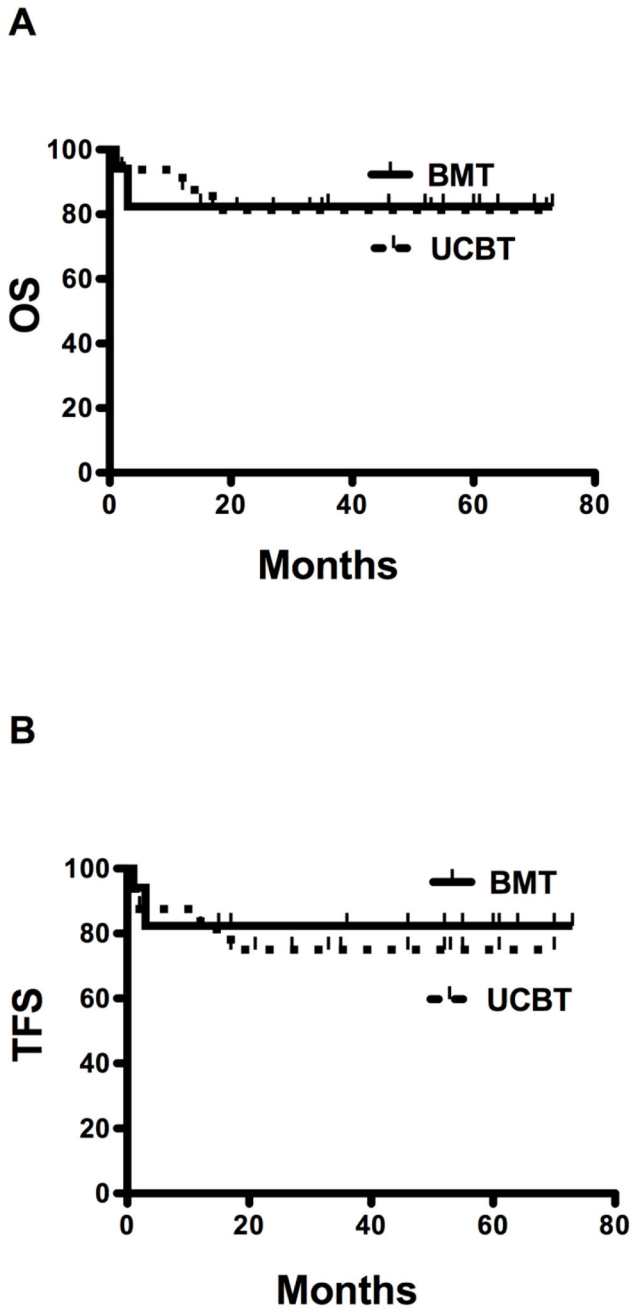


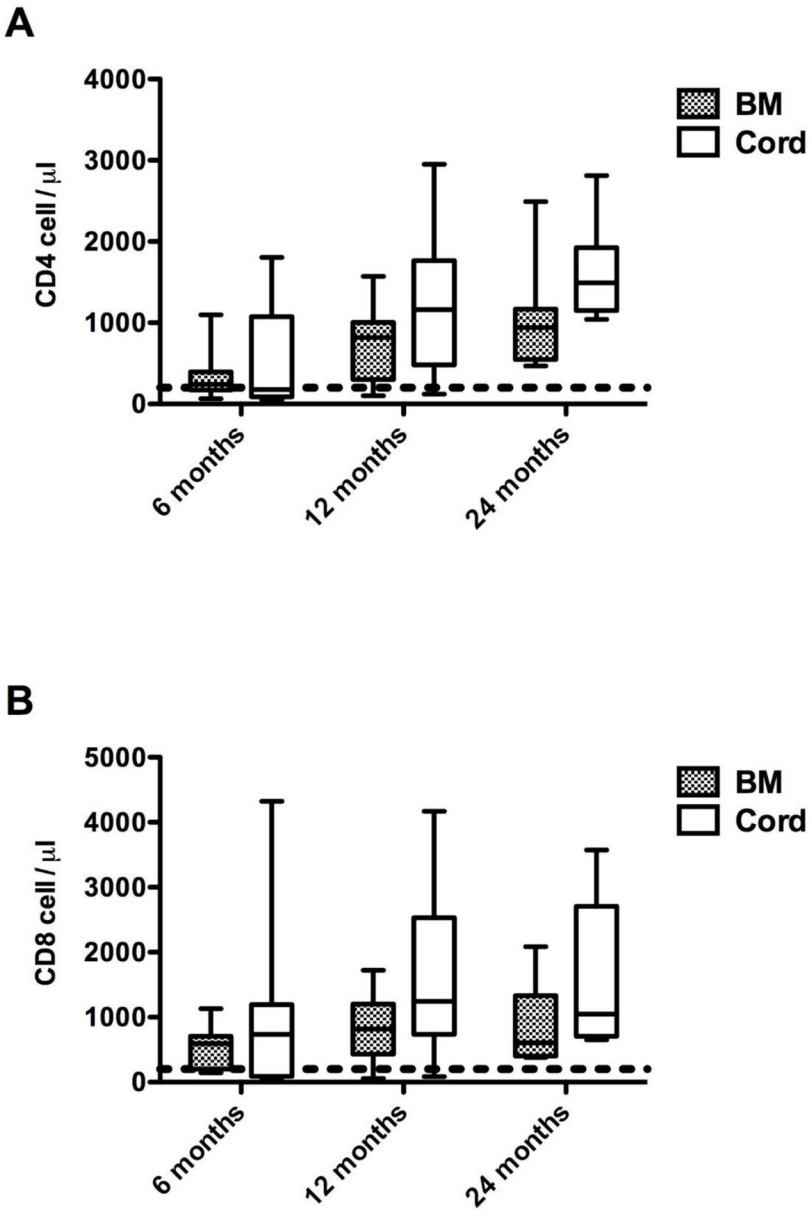
Figure 1. Kaplan–Meier Estimates of Survival after unrelated donor transplantation in pediatric patients with thalassemia

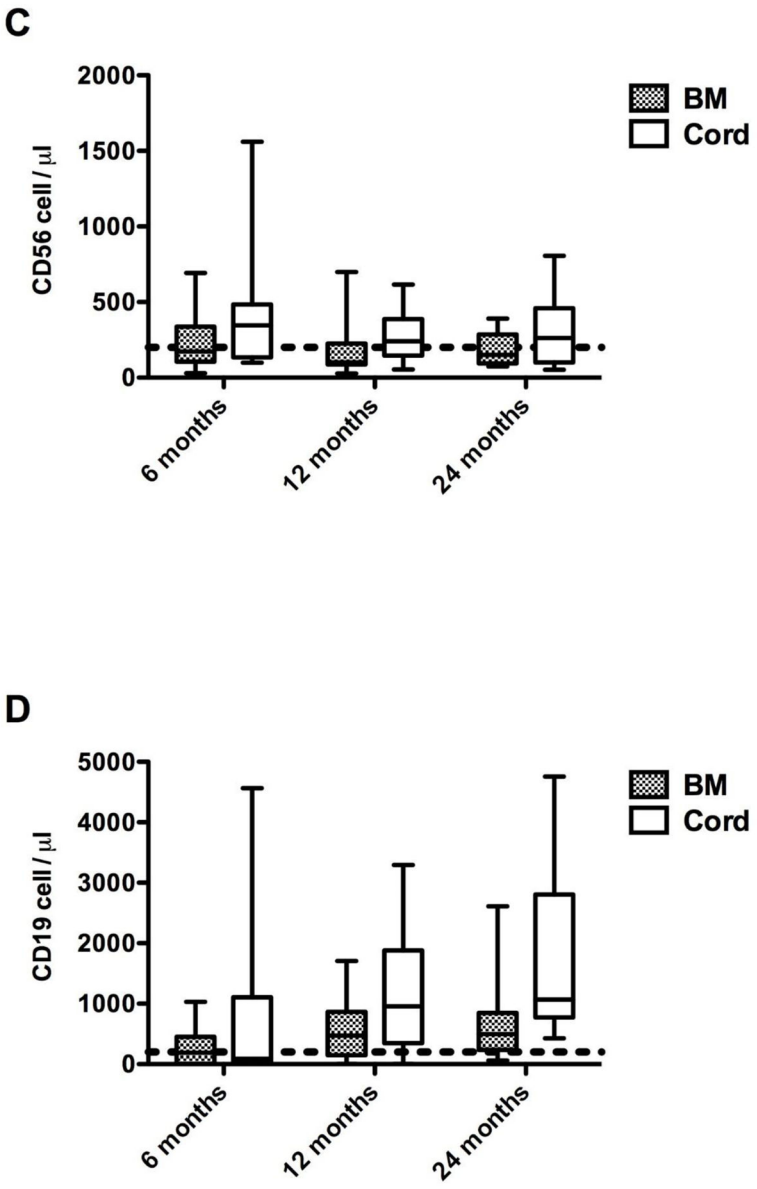
Figure 1A: Overall Survival (OS) 82.4% (95% CI 0.55–0.95) after marrow and 81.3% (95% CI 0.54–0.95) after cord blood transplantation

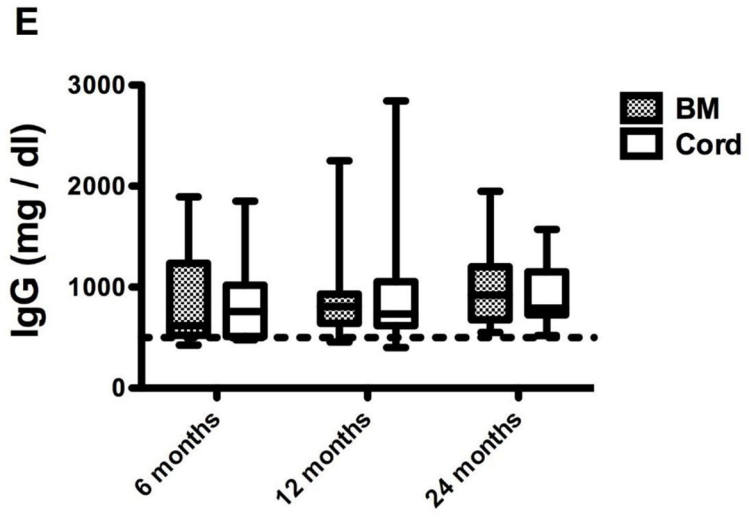
Figure 1B: Thalassemia- free Survival (TFS) 82.4% (95% CI 0.55–0.95) after marrow and 75% (95% CI 0.47–0.91) after cord blood transplantation

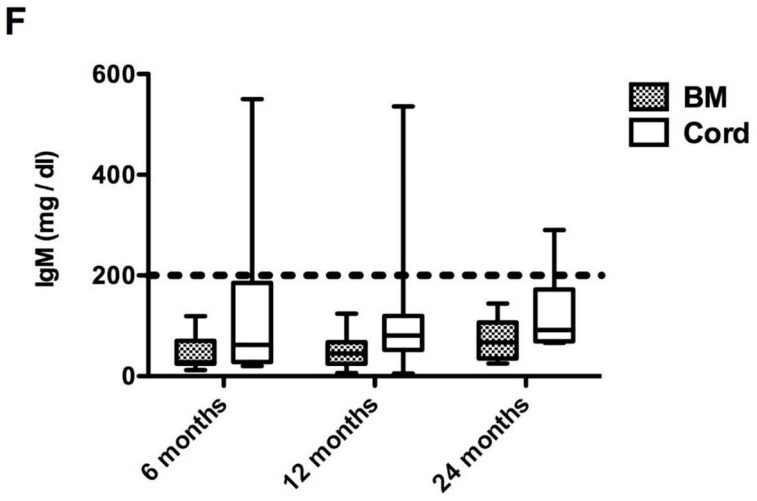
Overall Survival after matched unrelated donor bone marrow were similar when compared with that of cord recipients (relative risk [RR] = 0.99, 95% confidence interval [CI], 0.2–4.96; $P = .992$.

Thalassemia-free Survival after matched unrelated bone marrow (BM) recipients were similar when compared with that of cord recipients (relative risk [RR] = 0.7, 95% confidence interval [CI], 0.16–3.12; $P = .6$.









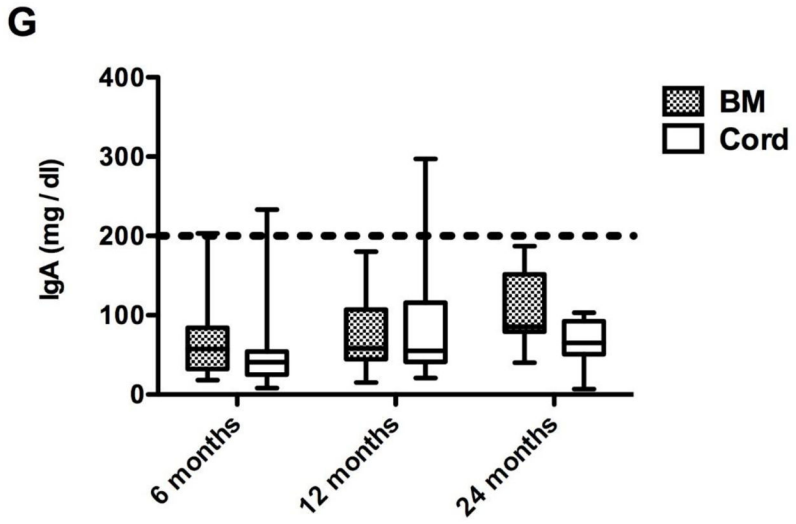


Figure 2. Immune reconstitution at 6, 12 and 24 months after unrelated donor transplantation in pediatric patients with thalassemia

Normal values are indicated with horizontal interrupted lines. The X-axis indicates time after transplant at assessment. The Y-axis indicates absolute lymphocyte numbers per mL for lymphocyte subpopulations and mg/dL for immunoglobulin levels. Recovery after marrow and cord transplants are indicated by box and whisker plots to display the median, 25th, and 75th percentile of the distribution (box); whiskers extend to the most extreme data points. Shaded boxes indicate levels after marrow and clear boxes after cord transplants.

Figure 2A: CD4+ T cells

Figure 2B: CD8+ T cells

Figure 2C: CD56+ NK cells

Figure 2D: CD19+ B cells

Figure 2E: IgG levels

Figure 2F: IgM levels

Figure 2G: IgA levels

Compared with marrow, cord blood recipients had significantly faster recovery with higher absolute numbers of CD4+, CD8+, CD56+ and CD19+ circulating lymphocytes in peripheral blood (p values 0.0025, 0.004, 0.02 and 0.002 respectively). IgM levels also recovered faster after cord blood transplantation (p=0.003). There was no difference in rate of recovery of IgG and IgA levels.

Table I

Donor and recipient characteristics

	Marrow	Cord Blood
Number	17	16
Sex(% male)	47%	56%
Median Recipient Age (years)(range)	10 (3–17)	3.5 (1–15)
Median TNC/Kg (10^8) infused (range)	4.38 (0.6–7.75)	0.6 (0.37–1.07)
Median CD34/kg (10^6) infused (range)	4.99 (2.89–10.77)	0.41 (0.1–1.7)
*HLA-matched donor	17	1
*HLA-mismatched donor	0	15
Median ferritin level (ng/ml) (range)	1104 (421–3024)	1352 (584–3199)
Hepatic fibrosis	7 mild 1 moderate	3 mild 1 moderate
Sex mismatch donor/recipient	6	5
ABO mismatch donor/recipient	9	11
CMV status		
Donor and recipient CMV+	7	0
Donor- recipient CMV+	4	13
Donor+ recipient CMV−	2	0
Donor and recipient CMV−	4	3
Pesaro risk classification		
Class 1	5	7
Class 2	12	7
Class 3	-	2

*see methods section for HLA-match criteria based on stem cell source

TNC= Total Nucleated Cells; HLA=Human Leucocyte Antigen

Table II

Summary of outcomes

	BM(n=17)	CB(n=16)	p
Follow-up months, median (range)	52 (7–79)	40 (8–78)	
Neutrophil engraftment, median(range)	13 days (10–25)	23 days (12–46)	<0.0001
Platelet engraftment, median(range)	24 days (18–49)	50 days (31–234)	<0.0048
Graft rejection	0	1 (6%)	
Cumulative incidence of aGVHD at day100			
II–IV	4 (24%)	7 (44%)	0.22
III–IV	3 (18%)	6 (38%)	
Cumulative incidence of cGVHD at 2 years	3 (21%)	4 (29%)	0.9
Limited Extensive	4 (29%)	3 (21%)	
Off immunosuppression @ 2 years	10 (71%)	11 (91%)	0.66
Lansky performance score at last follow-up	100 (86%)	100 (90%)	
	90 (7%)		
	80 (7%)	80 (10%)	
Viral Infection			
Adenovirus infection	2 (12%)	7 (44%)	
CMV reactivation	9 (53%)	13 (81%)	
EBV reactivation	7 (41%)	2 (12%)	
Hepatic sinusoidal obstruction syndrome	0	2 (12%)	
Transplant related mortality	3 (18%)	3 (19%)	0.9
Cause of death			
GVHD/Infection	1 (6%)	2 (13%)	
Diffuse alveolar hemorrhage	1 (6%)	0	
Viral pneumonitis and pulmonary hemorrhage	1 (6%)	1 (6%)	

aGVHD=acute graft-versus-host disease; cGVHD=chronic graft-versus-host disease; BM=Bone marrow; CB=Cord blood; CMV=cytomegalovirus; EBV=Epstein-Barr Virus