ASBMT American Society for Blood and Marrow Transplantation

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)

Naynesh R. Kamani,[1] Mark C. Walters,[2] Shelly Carter,[3] Victor Aquino,[4] Joel A. Brochstein,[5] Sonali Chaudhury,[6] Mary Eapen,[7] Brian M. Freed,[8] Michael Grimley,[9] John E. Levine,[10] Brent Logan,[7] Theodore Moore,[11] Julie Panepinto,[7] Suhag Parikh,[12] Michael A. Pulsipher,[13] Jane Sande,[1] Kirk R. Schultz,[14] Stephen Spellman,[15] Shalini Shenoy[16]

The Sickle Cell Unrelated Donor Transplant Trial (SCURT trial) of the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) is a phase II study of the toxicity and efficacy of unrelated donor hematopoietic cell transplantation in children with severe sickle cell disease (SCD) using a reduced-intensity conditioning regimen. Here we report the results for the cord blood cohort of this trial. Eight children with severe SCD underwent unrelated donor cord blood transplantation (CBT) following alemtuzumab, fludarabine, and melphalan. Cyclosporine or tacrolimus and mycophenolate mofetil were administered for graft-versus-host disease (GVHD) prophylaxis. Donor/recipient HLA match status was 6 of 6 (n = 1) or 5 of 6 (n = 7), based on low/intermediate-resolution molecular typing at HLA -A, -B, and high-resolution typing at -DRB1. Median recipient age was 13.7 years (range: 7.4-16.2 years), and median weight was 35.0 kg (range: 25.2-90.2 kg). The median precryopreservation total nucleated cell dose was 6.4×10^7 /kg (range: 3.1-7.6), and the median postthaw infused CD34 cell dose was 1.5×10^5 /kg (range: 0.2-2.3). All patients achieved neutrophil recovery (absolute neutrophil count $>500/mm^3$) by day 33 (median: 22 days). Three patients who engrafted had 100% donor cells by day 100, which was sustained, and 5 patients had autologous hematopoietic recovery. Six of 8 patients had a platelet recovery to $>50{,}000/mm^3$ by day 100. Two patients developed grade II acute GVHD. Of these, 1 developed extensive chronic GVHD and died of respiratory failure 14 months posttransplantation. With a median follow-up of 1.8 years (range: 1-2.6), 7 patients are alive with a 1-year survival of 100%, and 3 of 8 are alive without graft failure or disease recurrence. Based upon the high incidence of graft rejection after unrelated donor CBT, enrollment onto the cord blood arm of the SCURT trial was suspended. However, because this reduced-intensity regimen has demonstrated a favorable safety profile, this trial remains open to enrollment for unrelated marrow donor transplants. Novel approaches aimed at improving engraftment will be needed before unrelated CBT can be widely adopted for transplanting patients with severe SCD.

Biol Blood Marrow Transplant 18: 1265-1272 (2012)

KEY WORDS: Cord blood, Unrelated donor, Transplantation, Children, Sickle cell disease

INTRODUCTION

Several studies have shown that hematopoietic cell transplantation (HCT) for sickle cell disease (SCD) using HLA-identical sibling donors has curative potential, including a National Institutes of Health–supported multicenter prospective trial that

From the [1]Children's National Medical Center, Washington, DC; [2]Children's Hospital of Oakland, Oakland, California; [3]The EMMES Corporation, Rockville, Maryland; [4]Children's Medical Center of Dallas, Dallas, Texas; [5]Steven and Alexandra Cohen Children's Medical Center of New York, New Hyde Park, New York; [6]Children's Memorial Hospital, Chicago, Illinois; [7]Medical College of Wisconsin, Milwaukee, Wisconsin; [8]Clinimmune Labs, Aurora, Colorado; [9]Texas Transplant Institute, San Antonio, Texas; [10]University of Michigan, Ann Arbor, Michigan; [11]Mattel Children's Hospital at UCLA, Los Angeles, California; [12]Duke University, Durham, North Carolina; [13]University of Utah School of Medicine, Salt Lake City, Utah; [14]BC Children's Hospital, Vancouver, BC, Canada; [15]Center for International Blood and Marrow Transplant Research, Minneapolis, Minnesota; and [16]Washington University in St. Louis, St. Louis, Missouri.

Financial disclosure: See Acknowledgments on page 1271.

Correspondence and reprint requests: Naynesh R. Kamani, MD, Children's National Medical Center, 111 Michigan Avenue NW, Washington, DC 20010 (e-mail: NKamani@cnmc.org).

Received January 3, 2012; accepted January 29, 2012

1083-8791/$36.00
doi:10.1016/j.bbmt.2012.01.019

demonstrated an event-free survival (EFS) probability of 85% in children following a myeloablative preparative regimen of busulfan, cyclophosphamide, and antithymocyte globulin [1]. In addition, long-term follow-up studies from this and other patient series have demonstrated cessation of SCD-related events after successful HCT, even among those with mixed donor-host hematopoietic chimerism [2-5]. However, these observations have done little to expand the application of HCT for SCD because fewer than 20% of individuals have HLA-identical sibling donors and because there are ongoing concerns about the toxicity of this therapy. To address these challenges, a prospective multicenter, phase II clinical trial of HCT was initiated using a reduced-intensity conditioning regimen and well-matched unrelated donor cells. The overarching goal of this ongoing trial is to assess the toxicity and efficacy of such an approach and to expand the availability of HCT so that more individuals with severe SCD might benefit from this treatment option.

The hypothesis for this reduced-intensity regimen is the premise that host immune ablation can support successful donor cell engraftment without myeloablation. The trial was developed based on the results of a preliminary study of reduced-intensity HCT in 16 children with nonmalignant disorders who received alemtuzumab, fludarabine, and melphalan before HLA-identical sibling or unrelated donor HCT [6,7]. Graft rejection and graft-versus-host disease (GVHD) rates were low, and prompt engraftment occurred after transplantation from both related and unrelated donors. No late graft rejection was noted despite the early cessation of postgrafting immunosuppression. The regimen-related toxicity observed was early and related primarily to infections associated with this highly immunosuppressive conditioning regimen. This experience was distinctly different from the very high rate of graft rejection that was observed in several small series after nonmyeloablative HCT for SCD [8,9], and in another recent trial of nonmyeloablative matched sibling donor transplantation for adults with SCD where postgrafting immunosuppression had to be extended indefinitely to prevent late graft rejection [10]. Thus, we reasoned that our reduced-intensity conditioning approach might be sufficiently immunoablative to suppress host-versus-graft rejection and promote engraftment of donor cells after unrelated donor HCT in SCD. With careful attention to infection prevention and preemptive treatment of viral reactivation, it was also our aim to reduce the short- and long-term toxicity of transplantation.

The preliminary results of the ongoing phase II trial strongly suggest that this is a safe approach to unrelated donor HCT for SCD. However, we have observed a high rate of graft rejection after unrelated donor cord blood transplantation (CBT), which has resulted in closure of the cord blood arm of the study and caused us to look more carefully at factors that may have contributed to graft rejection. Here, we present the preliminary results for the cord blood cohort of this multicenter trial, which continues to enroll patients with 8 of 8 HLA-allele matched unrelated bone marrow donors.

STUDY DESIGN AND METHODS

Study Design

The Sickle Cell Unrelated Donor Transplant Trial (SCURT) is a phase II multicenter trial designed to estimate the efficacy and toxicity of unrelated donor HCT using a reduced-intensity conditioning regimen in children with SCD and high-risk features. It was initiated in 2008 and included 2 cohorts based on the donor hematopoietic stem cell source. The primary objective of the study was to determine EFS at 1 year after unrelated donor transplantation using either bone marrow or cord blood as a stem cell source. The secondary objectives included the determination of the effects of HCT on clinical and laboratory manifestations of SCD and the incidence of HCT complications such as transplant-related toxicity, graft failure, and acute and chronic GVHD (aGVHD, cGVHD). Stopping rules for the study included unacceptable rates of mortality, GVHD, or graft rejection.

Patients

The protocol was approved by the institutional review board at each of the 7 institutions that enrolled patients and by the institutional review board of the National Marrow Donor Program. Consent was obtained from legal guardians for all patients before enrollment; assents were obtained where appropriate. In addition, before enrollment, an independent external review committee approved enrollment by ensuring that the patient met disease-related eligibility criteria. An important eligibility criteria design consideration was to enroll only patients with SCD manifestations associated with poor long-term outcomes despite optimal supportive care. For the patients described in this report, eligibility included recipients 3-16 years of age with severe SCD with 1 or more of the following: (1) a clinically significant neurologic event (overt stroke) defined as any neurologic defect lasting >24 hours and accompanied by an infarct on cerebral magnetic resonance imaging; or, (2) a minimum of 2 episodes of acute chest syndrome (ACS) within the preceding 2-year period defined as new pulmonary alveolar consolidation involving at least 1 complete lung segment (associated with acute symptoms including fever, chest pain, tachypnea, wheezing, rales, or cough that is not attributed to

asthma or bronchiolitis) despite adequate supportive care measures; or, (3) a history of 3 or more severe pain events or vaso-occlusive crises (VOC) per year in the 2 years before enrollment. An additional criterion for patients with recurrent ACS or VOC was the requirement that they either fail to significantly benefit from or were unable or unwilling to continue supportive care therapy that included hydroxyurea.

Patients with HLA-matched sibling donors, HIV seropositivity, Lansky performance score <50, or uncontrolled bacterial, viral, or fungal infections were ineligible. Multiorgan assessment performed before enrollment was required to demonstrate the following: serum creatinine <1.5 upper limit of normal for age and glomerular filtration rate >100 mL/min/1.73 m^2; alanine aminotransferase and aspartate aminotransferance <5 times upper limits of normal and direct serum bilirubin <2 × upper limit of normal; left ventricular ejection fraction >40% or left ventricular shortening fraction >26%; and carbon monoxide diffusing capacity >40% of predicted (corrected for hemoglobin). For patients who had received regular blood transfusions for over a year and whose serum ferritin was >1000 ng/mL, a liver biopsy had to demonstrate the absence of cirrhosis or bridging fibrosis. The Hb S percentage had to be ≤45% 7 days before initiation of alemtuzumab. Iron chelation and/or hydroxyurea had to be discontinued 48 hours before alemtuzumab.

Donor Selection

The donor/recipient HLA match for umbilical cord blood transplantation (UCBT) was accomplished by low/intermediate-resolution molecular typing for class I HLA-A and -B alleles, and high-resolution molecular typing for HLA-DRB1 alleles (original HLA match). For the purpose of this report, high-resolution molecular typing for HLA-A, -B, -C, and -DRB1 alleles was retrospectively reviewed and reported as the final HLA match. The selected cord blood unit (CBU) was required to be ≥5 of 6 matched and provide a minimum of 3×10^7 total nucleated cells (precryopreservation) per kilogram (kg) of recipient weight.

Preparative Regimen

All patients received increasing doses of alemtuzumab intravenously daily for 3 days (10 mg, 15 mg, and 20 mg) starting on day −21 after a 3-mg intravenous (i.v.) test dose. Patients were discharged after alemtuzumab administration and readmitted to start fludarabine 30 mg/m^2 daily from day −8 to day −4. Melphalan (140 mg/m^2/day) was given on day −3 [6]. GVHD prophylaxis consisted of cyclosporine or tacrolimus and mycophenolate mofetil (MMF). Cyclosporine or tacrolimus was started on day −3 with regular monitoring to maintain therapeutic levels and continued to day 100, then tapered weekly and discontinued on day +180 if recipients had no evidence of GVHD. MMF (1 g i.v. every 8 hours for children ≥50 kg or 15 mg/kg i.v. every 8 hours for children <50 kg) was started on day −3 and given through day +45 or 7 days after engraftment, whichever was later. Monitoring of MMF levels was not mandated by the protocol. Granulocyte colony stimulating factor, 5 μg/kg/day, was started on day +7 and continued until neutrophil engraftment.

Endpoint Definitions

The primary endpoint of the study was EFS at 1 year posttransplantation.

Primary or late graft rejection, disease recurrence, or death was considered an event for this endpoint. Primary graft rejection was defined as the presence of <20% donor cells as assessed by bone marrow or peripheral blood chimerism assays on day 42 or the infusion of a second stem cell product on or before day 42. Late graft rejection was defined as the presence of <20% donor-derived hematopoietic cells in peripheral blood or bone marrow after day 42 in a patient with prior evidence of >20% donor cells or the infusion of a second stem cell product beyond day 42. Survival was defined as the time from transplantation to day of death or day of last follow-up. Neutrophil recovery was defined as the first day of achieving an absolute neutrophil count of at least 500/mm^3 for 3 consecutive days. Platelet recovery was defined as the first day of a minimum of 3 consecutive measurements on different days that the patient had achieved a platelet count >50,000/mm^3, and was platelet transfusion independent for a minimum of 7 days. The time to neutrophil or platelet recovery was defined as the time from transplantation to the first day of engraftment.

Acute GVHD assessments were made every 7 days to day 100 post-CBT, 6 months, 1 year, and 2 years after CBT. The grading of aGVHD followed the GVHD consensus grading scheme and methods of Weisdorf et al. [11]. Clinically significant infections were reported posttransplantation by site of infection, organism, and severity. Readmissions after the initial discharge following transplantation were reported by date and primary and secondary reasons for admission. The Common Terminology Criteria for Adverse Events version 3.0 was used to report expected grade 3-5 adverse events through 1-year posttransplantation. Maximum Common Terminology Criteria for Adverse Events grades across all organ systems were reported monthly for 3 months and at 6 and 12 months posttransplantation.

A truncated sequential probability ratio test for a binomial outcome was used to monitor for each of the 3 key safety endpoints, namely overall mortality,

Table 1. Patient Demographics

Patient ID	Gender	Race	Age at Transplantation (Years)	Disease Type	Lansky Score	Indication for Transplantation
02376	F	Black/AA	11.2	Hgb SS	80	VOC
02395	F	Black/AA	16.3	Hgb SS	80	Stroke
02497	M	Black/AA	14.3	Hgb SS	80	ACS
02541	F	Black/AA	7.4	Hgb SS	100	ACS
02611	M	Black/AA	13.9	Hgb Sßo thal	100	VOC
02613	F	Black/AA	10.9	Hgb SS	100	ACS
02760	F	White Hispanic	15.0	Hgb Sß+ thal	100	VOC
02901	F	Black/AA	13.6	Hgb SS	100	Stroke

graft rejection, and GVHD. The stopping rules were triggered if there was significant evidence that the day 100 overall mortality rate was >15% based on the truncated sequential probability ratio test, or if the day 100 graft rejection rate exceeded 20%, or if the day 100 grade III-IV GVHD rate exceeded 15%.

Anti-HLA Donor-Specific Antibodies (DSA)

A retrospective analysis for the presence of anti-HLA DSA was performed in 6 recipients using stored pretransplantation sera. Donor mismatched HLA alleles were identified and recipient DSA response was screened with the LABScreen® Single Antigen HLA Class I/II Antibody Detection system that uses microbeads coated with purified Class I or Class II HLA antigens for the detection of Class I or Class II HLA antibodies in the patient's plasma. Each bead in a LABScreen Single Antigen assay is specific for a single HLA antigen, permitting assessment of specificity for mismatched antigens carried by the donor.

Statistical Analysis

Survival estimates were calculated using the Kaplan-Meier method. Neutrophil and platelet recoveries, aGVHD and cGVHD, and graft rejection/disease relapse were analyzed using the cause-specific failure probability method (or cumulative incidence) [12]. Because of the small sample size, only univariate analyses were performed.

RESULTS

Study Population

Eight patients were enrolled for UCBT at 7 transplant centers. Baseline characteristics are shown in Table 1. The median age was 13.8 years (range: 7.4-16.2). The primary diagnosis was Hemoglobin SS disease (n = 6), Hgb S/β^0 Thalassemia (n = 1), and Hgb S/β^+ Thalassemia (n = 1). The indication for CBT was stroke in 2 patients, recurrent ACS in 3 patients, and recurrent VOC in 3 patients. The median precryopreserved total nucleated cell (TNC) dose was 6.4×10^7/kg (range: 3.1-7.6), and the postthaw infused TNC and $CD34^+$ cell dose/kg were 4.5×10^7 (range: 2.1-6.3) and 1.5×10^5 (range: 0.2-2.3), respectively.

Results of donor–recipient blood groups and HLA matching are shown in Table 2. Based on original donor–recipient HLA typing, all 8 patients were matched at 5 of 6 HLA antigens. However, at the allele level high resolution molecular HLA typing for HLA-A, -B, -C, and -DRB1, all donor/recipient pairs were mismatched for 1 (n = 2), 2 (n = 4), or 3 (n = 2) alleles. Of note, both patients who were allele-matched at the C locus engrafted, whereas 5 of 6 mismatched at 1 or both C loci rejected their grafts. The retrospective analysis for the presence of anti-HLA donor-specific antibodies failed to detect DSA in any of the 6 pretransplantation serum samples tested.

Engraftment and Survival

All 8 patients achieved neutrophil recovery with an absolute neutrophil count >500/mm^3 at a median of 22 days (range: 13-33) after CBT. Six of 8 patients had platelet recovery to 50,000/mm^3 by day 100. In 5 patients, blood count recovery was the result of graft rejection and autologous hematopoietic reconstitution, as demonstrated by 100% recipient DNA in chimerism studies performed on day 42 samples. No late graft rejections were observed, and the remaining 3 patients had sustained donor engraftment through last follow-up. Because of primary graft rejection, the 1-year EFS was 37.5% (95% confidence interval: 8.7%-67.4%). All graft rejections were nonlethal and were not characterized by marrow aplasia. With a median follow-up of 1.8 years (1-2.6), 1-year survival was 100%. The solitary death on this study occurred on day 430 from complications of cGVHD (Table 3).

Acute GVHD and cGVHD

The maximum GVHD grade was grade II (n = 2). An additional patient developed a limited skin rash (grade I). There was 1 case of chronic extensive GVHD.

Regimen-Related Toxicity

Serious, but reversible, transplant-related neurologic toxicities in the first 100 days developed in

Table 2. Donor–Recipient Characteristics

Patient ID	Original HLA Match	Donor–Recipient Allele Level HLA Match	Prior Chronic Transfusion Therapy	Recipient Blood Group	Donor Blood Group	Red-Cell Alloantibody	Antidonor-Specific HLA Antibodies
02376	5/6	7/8	Yes	B +ve	A +ve	Yes	No
02395	5/6	6/8	Yes	A +ve	A +ve	No	No
02497	5/6	5/8	Yes	A +ve	O -ve	Yes	Not tested
02541	5/6	6/8	No	O +ve	O +ve	Yes	No
02611	5/6	6/8	No	O +ve	B +ve	No	No
02613	5/6	6/8	No	A +ve	B +ve	No	No
02760	5/6	7/8	No	AB +ve	O +ve	No	No
02901	5/6	5/8	Yes	B +ve	O +ve	No	Not tested

2 patients. One patient, with hypertension, experienced a grade 4 intraventricular hemorrhage with seizures on day 7. A second patient developed posterior reversible leukoencephalopathy syndrome because of cyclosporine. Both patients experienced complete recovery. Two patients had grade 4 liver toxicity, which was transient. No patient developed idiopathic pneumonia syndrome or hepatic VOC disease. One patient developed Epstein-Barr virus reactivation with posttransplantation lymphoproliferative disease and a brain abscess in the context of cGVHD, and eventually died on day 430 from respiratory failure secondary to pneumonia.

There were a total of 19 infections (bacterial and viral) reported in 6 of 8 patients, with no infections reported in 2 patients. These included 9 episodes of bacteremia (8 Gram-positive organisms, 1 *Klebsiella*), 3 patients with cytomegalovirus viremia, 1 with Epstein-Barr virus reactivation progressing to posttranplantation lymphoproliferative disorder, 3 with upper respiratory tract infections (1 adenovirus, 1 parainfluenza-3, and 1 *Pseudomonas*), 1 with lower respiratory tract parainfluenza-3 infection, 1 adenovirus gastroenteritis, and 1 *C. difficile* enterocolitis.

DISCUSSION

Although allogeneic HCT has curative potential for patients with severe SCD, there are 2 major barriers to making this therapy available to eligible patients: the absence of HLA identical sibling donors and concerns about the risk of mortality or treatment-related toxicities [13]. In order to circumvent these barriers, we initiated a clinical trial of a reduced-intensity preparative regimen before unrelated donor HCT for severe SCD, in particular, relying upon the use of cord blood as a source of hematopoietic cells for allogeneic transplantation in patients with hemoglobinopathies [14,15]. With cord blood as a source of hematopoietic cells, it was our expectation that the risk of GVHD would be lower compared with marrow-derived hematopoietic cells and that ≥5 of 6 matched CB units would be available for the vast majority of potential recipients [16,17]. In the first report of sibling donor CBT using a myeloablative regimen in 44 children with hemoglobinopathies, the EFS was 79% for thalassemia and 90% for SCD with no treatment-related mortality; all the treatment failures were related to graft rejection [18]. Thus, we reasoned that the risk of graft rejection/disease recurrence might pose a similar challenge after unrelated CBT and we applied stringent HLA matching and cell dose requirements in anticipation of this problem.

Despite these considerations, we observed a high incidence of graft rejection after CBT (5 of 8 recipients) that was accompanied by autologous recovery of sickle erythropoiesis by 6 weeks after transplantation. This experience is consistent with other reports of graft rejection following nonmyeloablative or reduced-intensity preparation before CBT, and suggests that further intensification in the preparative regimen may be necessary to ensure donor engraftment. An early report of unrelated CBT in 3 children with SCD included a single case of graft rejection followed by recovery of autologous hematopoiesis [19], and successful donor cell engraftment followed by GVHD in 2 of 3 children. In a subsequent retrospective review of a 4-center experience with unrelated cord blood transplantation in children with SCD that included 4 additional recipients (n = 7), 5 patients received a single CBU matched at 4 of 6 HLA-antigens and 2 were matched 5 of 6 HLA-antigens. All 3 patients who received a reduced-intensity regimen experienced graft rejection. Of the 4 who received myeloablative regimens, 3 engrafted and 2 developed grade III-IV aGVHD [20]. A recent retrospective survey of UCBT in 51 patients with hemoglobinopathies conducted by the Center for International Blood and Marrow Transplant Research with Eurocord and NY Blood Center participation observed primary graft failure in 7 of 16 recipients despite the use of myeloablative conditioning therapy in 4 of the 7 patients. A better disease-free survival among recipients who received CBT with a TNC content $\geq 5 \times 10^7$/kg was observed [21]. The notion that a larger cellular content might promote durable engraftment after CBT for hemoglobinopathies was also suggested in a report by Jaing et al. [22] of successful durable donor engraftment in 4 of 5 children with thalassemia major who

Table 3. Transplant Characteristics

Patient ID	Prethaw TNC Dose ($\times 10^7$/kg)	Posthaw TNC Dose ($\times 10^7$/kg)	Postthaw CD34+ Dose ($\times 10^5$/kg)	CMV Status	Intratransplantation Complications (Day 90)	Day to ANC >500	Day to Plt >50 K	Donor Chimerism Day +42	Acute GVHD	Chronic GVHD	Outcome
02376	7.4	6.1	1.7	Pos	Grade II GVHD	+19	+31	100% donor	Gr II day +28	None	Alive day +704
02395	3.1	2.1	0.2	Neg	Seizure with intraventricular hemorrhage; *C. difficile* infection	+29	NR	100% host (6% donor day +98; 0% donor day +170)	None	None	Graft rejection;
02497	3.7	2.5	0.5	Pos	CMV viremia *S. viridans* infection	+25	+36	100% host	None	None	Graft rejection
02541	6.9	5.5	1.7	Neg	None	+33	NR	100% host	None	None	Graft rejection; disease recurrence day +181
02611	6.0	4.7	1.5	Pos	CMV viremia *Streptococcus* infection	+22	+40	100% host	None	None	Graft rejection
02613	7.6	6.3	2.3	Pos	CMV viremia Grade II GVHD	+14	+47	100% donor	Gr II day +14	Extensive cGVHD day +393	Died on day +430
02760	3.4	2.5	1.0	Neg	Gemella infection	+13	+42	100% donor	None	None	Alive day +365
02901	6.7	4.3	Not done	Pos	Fever day 11, presumed engraftment syndrome	+29	+45	100% host	None	None	Graft rejection disease recurrence day +127

CMV indicates cytomegalovirus.

received dual unrelated cord blood units matched at 4 of 6 HLA-antigens. Thus, CBT from unrelated donors appears to present unique challenges to a successful outcome among recipients with hemoglobinopathies.

Another factor that might contribute to the high incidence of graft rejection that we and others have observed after CBT for SCD is donor–recipient HLA incompatibility. In a cohort of 269 patients with hematologic malignancies undergoing myeloablative bone marrow transplantation from mismatched related donors, there was a linear correlation between a higher rate of graft rejection and increasing donor HLA incompatibility with prior alloimmunization (as assessed by a positive crossmatch for antidonor lymphocytotoxic antibody) [23]. A nonengraftment/graft failure rate that approached 20% was observed in the largest trial of unrelated CBT (COBLT) in children with hematologic malignancies following a myeloablative regimen [24]. This was primarily attributed to cell dose and donor–recipient mismatching as a lower cell dose and a greater degree of HLA mismatching were associated with higher nonengraftment rates [25-28]. In patients with hemoglobinopathies, these risk factors are probably amplified by the effect of multiple transfusion exposures that might sensitize the recipient to donor alloantigens. In addition, the cytokine milieu of SCD, which is one of inflammation and immune activation at the baseline, might also promote a host-versus-graft reaction and thereby interfere with engraftment even after myeloablative preparation [29]. Although it is possible this risk might be overcome by employing more immunosuppressive preparative regimens by the addition of either antithymocyte globulin or low-dose total body irradiation as reported in multiply transfused patients with aplastic anemia [30-32], we found that even the use of a highly immunosuppressive conditioning regimen was not sufficient to overcome these factors in patients with severe SCD.

Although donor–recipient ABO incompatibility can cause complications such as delayed red blood cell (RBC) engraftment, pure red cell aplasia, or hemolytic anemia, it is not associated with graft rejection in patients with hematologic malignancies [33]. However, RBC alloimmunization recently was reported as an independent predictor of HLA alloimmunization [34]. The RBC alloimmunization frequency approaches 25% of SCD patients in the absence of extended phenotype matching of RBC transfusions and thus might have contributed to graft rejection in this series. A retrospective analysis of archived pretransplantation sera from unrelated donor HCT recipients showed that the presence of donor-directed, HLA-specific alloantibodies was significantly associated with graft failure [35]. A similar recent analysis of sera from 386 myeloablative UCBT recipients showed that the presence of donor-specific antibodies correlated with

significantly lower neutrophil recovery compared with those who lacked alloantibodies [36]. The presence of DSA has also been shown to predict outcomes in double umbilical CBT with higher graft failure and 100 day mortality in those with preformed DSA [37]. We were unable to demonstrate the presence of HLA-specific DSA in any of the 6 patients in whom stored pretransplantation serum samples were available for testing.

van Rood et al. [38] analyzed the impact of administering a cord blood unit that had a noninherited maternal HLA antigen (NIMA), which was shared with a mismatched HLA antigen in the cord blood donor among patients with hematologic malignancies treated by UCBT. They showed that having a donor with a NIMA-shared HLA antigen resulted in lower transplant-related mortality, and speculated that this was related to improved neutrophil recovery especially in those who received a low TNC cell dose. Thus, in the future, it might be important to perform screening for HLA-directed alloantibodies and use novel donor selection criteria to improve the results of CBT for SCD.

We speculate that a number of modifications should be explored to improve the rate of engraftment after UCBT for severe SCD. The use of a myeloablative regimen might improve outcomes by overcoming host barriers to engraftment, but this effect can also cause more short- and long-term toxicity including marrow aplasia in the setting of a graft rejection. Alternatively, we have observed very little toxicity with the regimen currently employed in the SCURT trial, and thus have considered modifications that might not significantly alter the toxicity profile while increasing the immunosuppressive intensity of the regimen. The addition of hydroxyurea well before HCT to eradicate thalassemic clones and/or the addition of thiotepa to the preparative regimen resulted in lower rejection rates after HLA-identical sibling bone marrow transplantation in advanced thalassemia [39,40] and is currently being studied in a reduced intensity study of unrelated CBT for thalassemia. A higher TNC dose might also improve engraftment [21]. This might also be accomplished by the use of dual CBU transplantation; however, this approach has not been validated in the context of a prospective clinical trial [22,41,42]. Whenever possible, the selection of CB units that are negative for antigens against which there are preexisting recipient antibodies should be pursued. At the present time, the role of NIMA matching in impacting engraftment in UCBT is unclear and requires additional investigation. In summary, because the less stringent need for HLA matching in CBT might still expand HCT access to many SCD patients who might not otherwise be able to pursue HCT, strategies to overcome engraftment barriers in CBT should be pursued and tested in clinical trials.

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of Nancy Poland and Jeffrey Chell, MD, who provided support to the conduct of this trial, and to Nancy DiFronzo, PhD, who provided support to the conduct of this trial and helpful suggestions to the preparation of the manuscript. We thank the patients and parents who consented to participation.

Financial disclosure: This BMT CTN trial is supported in part by grant #U01HL069294 from the National Heart, Lung, and Blood Institute and the National Cancer Institute, and by the National Marrow Donor Program, NIH's National Center of Minority Health and Health Disparities, and the NHLBI's Sickle Cell Disease Clinical Research Network.

AUTHORSHIP STATEMENT

N.R.K. and S.S. participated in the study design, data analysis and interpretation, and wrote the paper. M.W., S.L.C., J.E.B., M.E., J.E.L., S.P., J.P., M.P., and K.S. participated in the study design and revised the manuscript. B.R.L. participated in study design, data analysis and interpretation, performed statistical analysis, and revised the manuscript. S.S. and B.F. performed the DSA analysis. V.A., J.B., S.C., M.G., T.M., S.P., and J.S. enrolled patients and provided clinical care.

REFERENCES

1. Walters M, Patience M, Leisenring W, et al. Stable mixed hematopoietic chimerism after bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant*. 2001;7:665-673.
2. Bernaudin F, Socie G, Kuentz M, et al. Long-term results of myeloablative stem-cell transplantation to cure sickle cell disease. *Blood*. 2007;110:2749-2756.
3. Vermylen C, Cornu G, Ferster A, et al. Haematopoietic stem cell transplantation for sickle cell anemia: the first 50 patients transplanted in Belgium. *Bone Marrow Transplant*. 1998;22:1-6.
4. Walters M, Patience M, Leisenring W, et al. Bone marrow transplantation for sickle cell disease. *N Engl J Med*. 1996;335:369-376.
5. Eggleston B, Patience M, Edwards S, et al. Effect of myeloablative bone marrow transplantation on growth in children with sickle cell anaemia: results of the multicenter study of haematopoietic cell transplantation for sickle cell anaemia. *Br J Haematol*. 2007;136:673-676.
6. Shenoy S, Grossman W, DiPersio J, et al. A novel reduced-intensity stem cell transplant regimen for nonmalignant disorders. *Bone Marrow Transplant*. 2005;35:345-352.
7. Rao A, Kamani N, Filopovich A, et al. Successful bone marrow transplantation for IPEX syndrome after reduced intensity conditioning. *Blood*. 2007;109:383-385.
8. Iannone R, Casella J, Fuchs E, et al. Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. *Biol Blood Marrow Transplant*. 2003;9:519-528.
9. Horan J, Liesveld J, Fenton P, Blumberg N, Walters M. Hematopoietic stem cell transplantation for multiply transfused patients with sickle cell disease and thalassemia after low-dose

total body irradiation, fludarabine, and rabbit anti-thymocyte globulin. *Bone Marrow Transplant*. 2005;35:171-177.
10. Hsieh M, Kang E, Fitzhugh C, et al. Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. *N Engl J Med*. 2009;361:2309-2317.
11. Weisdorf D, Hurd D, Carter S, Howe C, Jensen L, Wagner J. Prospective grading of graft-versus-host disease after unrelated donor marrow transplantation: a grading algorithm versus blinded expert panel review. *Biol Blood Marrow Transplant*. 2003;9:512-518.
12. Gooley T, Leisenring W, Crowley J, Storer B. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
13. Walters M, Patience M, Leisenring W, et al. Barriers to bone marrow transplantation for sickle cell anemia. *Biol Blood Marrow Transplant*. 1996;2:100-104.
14. Kelly P, Kurtzberg J, Vichinsky E, Lubin B. Umbilical cord blood stem cells: application for the treatment of patients with hemoglobinopathies. *J Pediatr*. 1997;130:695-703.
15. Laughlin M. UCB allogeneic transplantation for hemoglobinopathies. *Blood*. 2003;101:2077.
16. Adamkiewicz T, Boyer M, Bray R, Haight A, Yeager A. Identification of unrelated cord blood units for hematopoietic stem cell transplantation in children with sickle cell disease. *J Pediatr Hematol Oncol*. 2006;28:29-32.
17. Krishnamurti L, Abel S, Maiers M, Flesch S. Availability of unrelated donors for hematopoietic stem cell transplantation for hemoglobinopathies. *Bone Marrow Transplant*. 2003;31:547-550.
18. Locatelli F, Rocha V, Reed W, et al. Related umbilical cord blood transplantation in patients with thalassemia and sickle cell disease. *Blood*. 2003;101:2137-2143.
19. Adamkiewicz T, Mehta P, Boyer M, et al. Transplantation of unrelated placental blood cells in children with high-risk sickle cell disease. *Bone Marrow Transplant*. 2004;34:405-411.
20. Adamkiewicz T, Szabolcs P, Haight A, et al. Unrelated cord blood transplantation in children with sickle cell disease: review of four-center experience. *Pediatr Transplant*. 2007;11:641-644.
21. 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;17:1375-1382.
22. Jaing T, Sun C, Lee W, Wen Y, Yang C, Hung I. Second transplant with two unrelated cord blood units for early graft failure after cord blood transplantation for thalassemia. *Pediatr Transplant*. 2009;13:766-768.
23. Anasetti C, Amos D, Beatty P, et al. Effect of HLA compatibility on engraftment of bone marrow transplants in patients with leukemia or lymphoma. *N Engl J Med*. 1989;320:197-204.
24. Kurtzberg J, Prasad V, Carter S, et al. Results of the Cord Blood Transplantation Study (COBLT): clinical outcomes of unrelated donor umbilical cord blood transplantation in pediatric patients with hematologic malignancies. *Blood*. 2008;112:4318-4327.
25. Gluckman E, Rocha V, Arcese W, et al. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. *Exp Hematol*. 2004;32:397-407.
26. Kamani N, Spellman S, Hurley C, et al. State of the art review: HLA matching and outcome of unrelated donor umbilical cord blood transplants. *Biol Blood Marrow Transplant*. 2008;14:1-6.
27. Rocha V, Gluckman E; Eurocord-Netcord registry and European Blood and Marrow Transplant group. Improving outcomes of cord blood transplantation: HLA matching, cell dose and other graft- and transplantation-related factors. *Br J Haematol*. 2009;147:262-274.
28. Barker J, Scaradavou A, Stevens C. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 cord blood recipients with hematologic malignancies. *Blood*. 2010;115:1843-1849.
29. 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;107:2984-2992.
30. Gale R, Ho W, Feig S, et al. Prevention of graft rejection following bone marrow transplantation. *Blood*. 1981;57:9-12.
31. Camitta B, Storb R, Thomas E. Aplastic anemia (first of two parts): pathogenesis, diagnosis, treatment, and prognosis. *N Engl J Med*. 1982;306:645-652.
32. Camitta B, Storb R, Thomas E. Aplastic anemia (second of two parts): pathogenesis, diagnosis, treatment, and prognosis. *N Engl J Med*. 1982;306:712-718.
33. Kanda J, Ichinohe T, Matsuo K, et al. Impact of ABO mismatching on the outcomes of allogeneic related and unrelated blood and marrow stem cell transplantations for hematologic malignancies: IPD-based meta-analysis of cohort studies. *Transfusion*. 2009;49:624-635.
34. McPherson M, Anderson A, Castillejo M, et al. HLA alloimmunization is associated with RBC antibodies in multiply transfused patients with sickle cell disease. *Pediatr Blood Cancer*. 2010;54:552-558.
35. Spellman S, Bray R, Rosen-Bronson S, et al. The detection of donor-directed, HLA-specific alloantibodies in recipients of unrelated hematopoietic cell transplantation is predictive of graft failure. *Blood*. 2010;115:2704-2708.
36. Takanashi M, Atsuta Y, Fujiwara K, et al. The impact of anti-HLA antibodies on unrelated cord blood transplantations. *Blood*. 2010;116:2839-2846.
37. Cutler C, Kim HT, Sun L, et al. Donor-specific anti-HLA antibodies predict outcome in double umbilical cord blood transplantation. *Blood*. 2011 Sep 22 [Epub ahead of print].
38. van Rood J, Stevens C, Smits J, Carrier C, Carpenter C, Scaradavou A. Reexposure of cord blood to noninherited maternal HLA antigens improves transplant outcome in hematological malignancies. *Proc Natl Acad Sci USA*. 2009;106:19952-19957.
39. Sodani P, Gaziev D, Polchi P, et al. New approach for bone marrow transplantation in patients with class 3 thalassemia aged younger than 17 years. *Blood*. 2004;104:1201-1203.
40. Hongeng S, Pakakasama S, Chuansumrit A, et al. Reduced intensity stem cell transplantation for treatment of class 3 Lucarelli severe thalassemia patients. *Am J Hematol*. 2007;82:1095-1098.
41. Barker J, Weisdorf D, DeFor T, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood*. 2005;105:1343-1347.
42. Brunstein C, Barker J, Weisdorf D, et al. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. *Blood*. 2007;110:3064-3070.