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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)

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Abstract

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 6/6 (n=1) or 5/6 (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 to 16.2 years), and median weight was 35.0 kg (range 25.2 to 90.2 kg). The median pre-cryopreservation total nucleated cell dose was 6.4×10^7 /kg (range 3.1 to 7.6) and the median post-thaw infused CD34 cell dose was 1.5×10^5 /kg (range 0.2 to 2.3). All patients achieved neutrophil recovery ($ANC > 500/mm^3$) by day 33 (median 22 days). Three patients who engrafted had 100% donor cells by day 100, which was sustained and five patients had autologous hematopoietic recovery. Six of eight patients had a platelet recovery to $> 50,000/mm^3$ by day 100. Two patients developed grade II acute GVHD. Of these, one developed extensive chronic GVHD and died of respiratory failure 14 months post-transplant. 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.

Conclusion—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, since 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.

Keywords

cord blood; unrelated donor; transplantation; children; sickle cell disease

INTRODUCTION

Several studies have shown that hematopoietic cell transplantation (HCT) for sickle cell disease using HLA-identical sibling donors has curative potential, including a NIH-supported multicenter prospective trial that demonstrated an event-free survival (EFS) probability of 85% in children following a myeloablative preparative regimen of busulfan, cyclophosphamide and anti-thymocyte globulin¹. In addition, long-term follow-up studies from this and other patient series have demonstrated cessation of sickle cell disease-related events after successful HCT, even among those with mixed donor-host hematopoietic chimerism²⁻⁵. However, these observations have done little to expand the application of HCT for sickle cell disease 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 over-arching 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 sickle cell disease 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 non-malignant disorders who received alemtuzumab, fludarabine and melphalan before HLA-identical sibling or unrelated donor HCT^{6,7}. Graft rejection and graft-versus-host disease 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 post-grafting 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 non-myeloablative HCT for sickle cell disease^{8,9}, and in another recent trial of non-myeloablative MSD transplantation for adults with SCD where post-grafting immunosuppression had to be extended indefinitely to prevent late graft rejection¹⁰. 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 sickle cell disease. With careful attention to infection prevention and pre-emptive 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 sickle cell disease. However, we have observed a high rate of graft rejection after unrelated donor 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 8/8 HLA-allele matched unrelated bone marrow donors.

STUDY DESIGN AND METHODS

Study Design

The SCURT trial is a Phase II multi-center 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 event-free survival at 1 year after URD transplant 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, acute and chronic GVHD. 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 IRB of the National Marrow Donor Program. Consent was obtained from legal guardians for all patients prior to enrollment; assents were obtained where appropriate. In addition, prior to 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 sickle cell disease 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 one 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 (MRI); OR, 2) a minimum of two episodes of acute chest syndrome (ACS) within the preceding 2-

year period defined as new pulmonary alveolar consolidation involving at least one 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 prior to 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 less than 50, or uncontrolled bacterial, viral or fungal infections were ineligible. Multi-organ assessment performed prior to enrollment was required to demonstrate the following: serum creatinine < 1.5 upper limit of normal for age and GFR > 100 ml/min/1.73 m²; ALT and AST < 5 times upper limits of normal and direct serum bilirubin < 2 × upper limit of normal; left ventricular ejection fraction > 40% or LV shortening fraction > 26%; and DLCO > 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 prior to initiation of alemtuzumab. Iron chelation and/or hydroxyurea had to be discontinued 48 hours prior to alemtuzumab.

Donor Selection

The donor/recipient HLA match for 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 CBU was required to be 5/6 matched and provide a minimum of 3×10^7 total nucleated cells (pre-cryopreservation) 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 test dose. Patients were discharged after alemtuzumab administration and readmitted to start fludarabine 30 mg/m² daily from day -8 to day -4. Melphalan (140 mg/m²/day) was given on day -3⁶. 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 gram IV q 8 hours for children ≥ 50 kg or 15 mg/kg IV q 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. G-CSF 5 µg/kg/day was started on day +7 and continued until neutrophil engraftment.

Endpoint Definitions

The primary endpoint of the study was event-free survival at 1 year post-transplant. 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 prior to 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 transplant to day of death or day of last follow up. Neutrophil recovery was defined as the first day of achieving an ANC of at least $500/\text{mm}^3$ for three consecutive days. Platelet recovery was defined as the first day of a minimum of three consecutive measurements on different days that the patient had achieved a platelet count $> 50,000/\text{mm}^3$, and was platelet transfusion independent for a minimum of seven days. The time to neutrophil or platelet recovery was defined as the time from transplant 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 acute GVHD followed GVHD consensus grading scheme and methods of Weisdorf et al¹¹. Clinically significant infections were reported post-transplant by site of infection, organism and severity. Re-admissions after the initial discharge following transplant were reported by date and primary and secondary reasons for admission. The Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 was used to report expected grade 3–5 adverse events through 1 year post-transplant. Maximum CTCAE grades across all organ systems were reported monthly for 3 months and at 6 and 12 months post-transplant.

A truncated sequential probability ratio test (SPRT) for a binomial outcome was used to monitor for each of the three key safety endpoints, namely overall mortality, graft rejection and GVHD. The stopping rules were triggered if there was significant evidence that the day 100 overall mortality rate was greater than 15% based on the truncated SPRT, 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

A retrospective analysis for the presence of anti-HLA donor specific antibodies (DSA) was carried out in 6 recipients using stored pre-transplant sera. Donor mismatched HLA alleles were identified and recipient DSA response was screened using 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, acute GVHD and chronic GVHD, and graft rejection/disease relapse were analyzed using the cause-specific failure probability method (or cumulative incidence, CINC)¹². Due to 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 to 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 pre-cryopreserved total nucleated cell (TNC) dose was $6.4 \times 10^7/\text{kg}$ (range 3.1 to 7.6), and the post thaw infused TNC and CD34+ cell dose per kg were 4.5×10^7 (range 2.1 to 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 one (n=2), two (n=4), or three (n=2) alleles. Of note, both patients who were allele matched at the C locus engrafted whereas 5/6 mismatched at one 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 six pre-transplant serum samples tested.

Engraftment and Survival

All eight patients achieved the neutrophil recovery with an ANC > 500/mm³ at a median of 22 days (range 13–33) after CBT. Six of eight patients had platelet recovery to 50,000/mm³ by day 100. In five 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 three patients had sustained donor engraftment through last follow-up. Due to primary graft rejection, the one-year event free survival was 37.5% (95% CI: 8.7 – 67.4%). All graft rejections were non-lethal and were not characterized by marrow aplasia. With a median follow-up of 1.8 years (1 to 2.6), one year survival was 100%. The solitary death on this study occurred on day 430 from complications of chronic GVHD.

Acute and Chronic GVHD

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

Regimen Related Toxicity

Serious, but reversible, transplant related neurological toxicities in the first 100 days developed in two patients. One patient, with hypertension, experienced a grade 4 intraventricular hemorrhage with seizures on day 7. A second patient developed posterior reversible leukoencephalopathy syndrome due to cyclosporine. Both patients experienced complete recovery. Two patients had grade 4 liver toxicity, which was transient. No patient developed idiopathic pneumonia syndrome or hepatic veno-occlusive disease. One patient developed EBV reactivation with post-transplant lymphoproliferative disease and a brain abscess in the context of chronic GVHD and eventually died on day 430 from respiratory failure secondary to pneumonia.

There were a total of 19 infections (bacterial and viral) reported in six of eight patients with no infections reported in 2 patients. These included 9 episodes of bacteremia (8 gram positive organisms, 1 Klebsiella), 3 patients with CMV viremia, 1 with EBV reactivation progressing to PTLD, 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 hematopoietic cell transplantation (HCT) has curative potential for patients with severe SCD, there are two 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 toxicities¹³. In order to circumvent these barriers, we initiated a clinical trial of a reduced intensity preparative regimen before unrelated donor HCT for severe sickle cell disease, 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 graft-vs-host disease would be lower compared to marrow-derived hematopoietic cells and that 5/6 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 rejection¹⁸. 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 non-myeloablative 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 hematopoiesis¹⁹, and successful donor cell engraftment followed by GVHD in 2 of 3 children. In a subsequent retrospective review of a four-center experience with unrelated cord blood transplantation in children with SCD that included 4 additional recipients (n=7), 5 patients received a single cord blood unit matched at 4/6 HLA-antigens and 2 were matched 5/6 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 acute GVHD²⁰. A recent retrospective survey of UCBT in 51 patients with hemoglobinopathies conducted by the CIBMTR with Eurocord and NY Blood Center participation observed primary graft failure in 7/16 recipients despite the use of myeloablative conditioning therapy in 4 of the 7 patients. A better disease-free survival among recipients that received CBT with a TNC content $5 \times 10^7/\text{kg}$ was observed²¹. 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 of successful durable donor engraftment in 4 of 5 children with thalassemia major who received dual unrelated CB units matched at 4/6 HLA-antigens²². 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 sickle cell disease is donor-recipient HLA incompatibility. In a cohort of 269 patients with hematologic malignancies undergoing myeloablative BMT 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 anti-donor lymphocytotoxic antibody)²³. A non-engraftment/graft failure rate that approached 20% was observed in the largest trial of unrelated CBT (COBLT) in children with hematologic malignancies following a myeloablative regimen²⁴. 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 non-engraftment rates²⁵⁻²⁸. 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 sickle cell disease, 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 preparation²⁹. While 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 anemia³⁰⁻³², we found that even the use of a highly immunosuppressive

conditioning regimen was not sufficient to overcome these factors in patients with severe sickle cell disease.

While donor recipient ABO incompatibility can cause complications such as delayed red blood cell engraftment, pure red cell aplasia or hemolytic anemia, it is not associated with graft rejection in patients with hematologic malignancies³³. However, RBC alloimmunization recently was reported as an independent predictor of HLA alloimmunization³⁴. 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 pre-transplant sera from unrelated donor HCT recipients showed that the presence of donor-directed, HLA-specific alloantibodies was significantly associated with graft failure³⁵. 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 as compared to those who lacked alloantibodies³⁶. 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 DSA³⁷. We were unable to demonstrate the presence of HLA-specific DSA in any of the six patients in whom stored pre-transplant serum samples were available for testing.

Van Rood et al analyzed the impact of administering a CB unit that had a non-inherited maternal HLA antigen (NIMA) that 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 dose³⁸. Thus, in the future, it might be important to perform screening for HLA directed allo-antibodies and use novel donor selection criteria to improve the results of CBT for sickle cell disease.

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-ID sibling BMT 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 engraftment²¹. This might also be accomplished by the use of dual cord blood unit 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 pre-existing 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.

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Table 1

Patient Demographics

Patient ID	Gender	Race	Age at transplant (yrs)	Disease Type	Lansky Score	Indication for Transplant
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 Sβo thal	100	VOC
02613	F	Black/AA	10.9	Hgb SS	100	ACS
02760	F	White Hispanic	15.0	Hgb Sβ+ thal	100	VOC
02901	F	Black/AA	13.6	Hgb SS	100	Stroke

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	Anti-donor 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

Table 3

Transplant Characteristics

Patient ID	Pre-Thaw TNC Dose ($\times 10^7/\text{kg}$)	Post-Thaw TNC Dose ($\times 10^7/\text{kg}$)	Post-Thaw CD34+ Dose ($\times 10^5/\text{kg}$)	CMV status	Intra-transplant Complications (Day 90)	Day to ANC > 500	Day to Ptt > 50K	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